Relation of Serum 25 Hydroxy Vitamin D3 Levels with Cardiovascular Risk Factors in Type 2 Diabetic Patients

Tip 2 Diyabetik Hastalarda Serum 25 Hidroksi Vitamin D3 Düzeyleri ile Kardiyovasküler Risk Faktörleri Arasındaki İlişki

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ABSTRACT Objective: Low serum levels of vitamin D are associated with diabetes mellitus, metabolic syndrome and cardiovascular disease. In this study it was aimed to investigate the role of 25 hydroxy vitamin D3 in some cardiovascular risk factors in diabetic patients. **Material and Methods:** Our study was conducted in 101 type 2 diabetic patients and 60 controls. Risk markers of cardiovascular disease and 25 hydroxy vitamin D3 were analysed and compared in two groups. **Results:** Significantly lower 25 hydroxy vitamin D3, significantly high body mass index, waist and hip circumference, fasting glucose and insulin, hemoglobin A1c, indirect insulin resistance index, systolic blood pressure, diastolic blood pressure, triglycerides, C-reactive protein (p:<0.01 all) were obtained in diabetic group, compared to controls, there were not any difference in homocysteine levels. In correlation analysis of diabetics negative correlations were found between 25 hydroxy vitamin D3 and body mass index (p:<0.001, r:-0.23), HbA1c (p:<0.05, r:-0.21), fasting insulin (p:<0.05, r:-0.01), indirect insulin resistance index (p:<0.05, r:-0.20), systolic blood pressure (p:<0.05, r:-0.30), diastolic blood pressure (p:<0.05, r:-0.40), and triglyceride (p:<0.05, r:-0.24). **Conclusion:** These results show that diabetic patients have lower 25 hydroxy vitamin D levels than controls. Diabetics having low 25 hydroxy vitamin D3 levels have higher glucose, triglyceride, blood pressure, insulin resistance, C-reactive protein and are more obese, but they do not have different homocysteine levels.

Key Words: Vitamin D; diabetes mellitus, type 2; cardiovascular diseases; obesity

ÖZET Amaç: Düşük serum vitamin D düzeyleri diabetes mellitus, metabolik sendrom ve kardiyovasküler hastalıklar ile ilişkilidir. Bu çalışmada 25 hidroksi vitamin D3'ün diyabetik hastalarda bazı risk faktörleri üzerine etkisinin araştırılması amaçlanmıştır. **Gereç ve Yöntemler:** Çalışmamız 101 tip 2 diyabetik hasta ve 60 kontrol kişiden oluştu. Kardiyovasküler hastalığa ait risk belirteçleri ve 25 hidroksi vitamin D3 düzeyleri iki grup içinde araştırıldı ve karşılaştırıldı. **Bulgular:** Diyabetik hastalarda kontrollerle kıyaslandığında belirgin olarak düşük 25 hidroksi vitamin D3, belirgin olarak yüksek vücut kitle indeksi, bel ve kalça çevresi, açlık kan şekeri ve insülin, hemoglobin A1c, indirekt insülin rezistans indeksi, sistolik ve diyastolik kan basıncı, trigliserid, C-reaktif protein saptandı (hepsi p:<0,01), homosistein değerleri arasında fark bulunamadı. Diyabetik hastalar arasındaki korelasyon analizinde 25 hidroksi vitamin D3 ile vücut kitle indeksi (p:<0,001, r:-0,23), HbA1c (p:<0,05, r:-0,21), açlık insülini (p:<0,05, r:-0,01), indirekt insülin rezistans indeksi (p:<0,05, r:-0,20), sistolik kan basıncı (p:<0,05, r:-0,30), diyastolik kan basıncı (p:<0,05, r:-0,40), ve trigliserid (p:<0,05, r:-0,24) arasında negatif korelasyon saptandı. **Sonuç:** Sonuçlarımız, diyabetik hastaların kontrollere oranla düşük 25 hidroksi vitamin D3 düzeylerine sahip olduklarını gösterdi. 25 Hidroksi vitamin D3 seviyelerine sahip olan diyabetik hastalar daha yüksek glukoz, trigliserid, kan basıncı, insülin rezistansı, C-reaktif protein seviyelerine sahip ve daha obezdiler, fakat farklı homosistein düzeylerine sahip değillerdi.

Anahtar Kelimeler: Vitamin D; diabetes mellitus, tip 2; kalp ve damar hastalıkları; obezite

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Serum concentration of 25-hydroxy vitamin D3(25(OH)D3) is the best indicator of vitamin D status.[1] In contrast to 25(OH)D3 circulating 1,25(OH)2D is generally not a good indicator of vitamin D status be-

cause it has a short half life of 15 hours and serum concentrations are closely regulated by PTH, Ca and P. 1,25(OH)2D do not decrease until vitamin D defeciency is severe.[2] Hypovitaminosis D was defined as serum 25(OH)D3 concentration <20ng/ml.[3,4]

The incidence of cardiovascular disease is increasing at an alarming rate both nationally and worldwide, with accounting for nearly 40% of worldwide all deaths each year.[5,6] The factors that make up the Framingham risk score (age, sex, blood pressure, serum total cholesterol or low density lipoprotein cholesterol, high density lipoprotein cholesterol levels, smoking and diabetes) account for most of the excess risk for incident cardiovascular disease(CVD).[7] Several lines of evidence have implicated some markers have received much attention as emerging risk factors that could account for some of the unexplained variability in CVD risk, such as C-recative protein (CRP) and homocysteine (Hcy).[8-13] Recently, there is accumulating evidence suggesting that altered vitamin D homeostasis may also play a role in cardiovascular disease as well as diabetes and metabolic syndrome.[14-18]

Keeping in mind these complex interactions between these cardiovascular risk factors and vitamin D, we examined the relation of vitamin D with obesity and insulin resistance markers, glucose and lipid metabolism, blood pressure, CRP and Hcy in patients with type 2 diabetes mellitus (DM) in a group of patients in Ankara where not so much investigations were made.

MATERIAL AND METHODS

PATIENTS:

One hundred and one DM patients, aged from 30-80 years, were recruited from the outpatient Clinic of Ankara Training and Research Hospital from January 2011 to February 2011. Sixty healthy age-matched people created the control group.

Subjects with chronic diseases of renal and liver, skin disorders, malabsorption, inflammatory bowel or Celiac disease (in history or nowadays), and ones taking medications that may interfere serum levels of 25(OH)D3 were excluded.

After detailed physical examination, in all subjects body weight and height were measured. Waist was measured when fasting, in standing position halfway between costal edge and iliac crest, whereas hip was measured at the greatest circumference around the buttocks, by a non elastic measure. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m2).

Systolic and diastolic blood pressure (SBP and DBP) were measured after a 5 min rest in the semi-sitting position with a sphygmomanometer. Blood pressure was determined at least three times at the right upper arm, and the mean was used in the analysis. The patients who were taking antihypertensive drugs or patients whose determined mean blood pressure levels ≥140/90 mmHg were diagnosed as hypertensive.19

Blood was withdrawn after 12 hour of overnight fasting, at 08.30 a.m. for fasting plasma glucose (FPG), glycated hemoglobin (HbA1c), fasting insulin (FI), serum total and high density lipoprotein cholesterol (HDL-C), triglycerides (TG), CRP, Hcy, Calcium (Ca), Phosphorus (P), parathyroid hormone(PTH) and 25 hydroxy vitamin D [25(OH)D3)] levels.

An indirect measure of insulin resistance was calculated from the fasting plasma insulin (μunite /ml)xfasting plasma glucose (mmol/l) /22.5 formula as homeostasis model assessment-insulin resistance (HOMA-IR).20

This study was performed according to the Helsinki decleration 2008. The local ethics committee approved this study and all the subjects gave written informed consent.

LABORATORY METHODS

Plasma glucose, total cholesterol, TG and HDL-C, Ca and P concentrations were determined by enzymocalorimetric spectrophotometric method in a Roche/Hitachi molecular PP autoanalyser. Low density lipoprotein cholesterol (LDL-C) was calculated by the Friedewald Formula (LDL: Total cholesterol-HDL-TG/5). Insulin was measured by means of DRG Diagnostics (DRG Instruments

GmbH, Germany) ELISA kits and FI was measured by TOSOH G7 HPLC system. PTH and TSH were determined with Advia Sentor XP device by chemoluminescence method. High sensitivity C-reactive protein (CRP) was measured by immunoflowmetric tests by Beckman-Cutler device. Hcy concentrations were determined according to the method of HPLC using Agilend 1100 device. PTH was determined with Advia Sentor XP device by chemoluminescence method.

For the measurements of 25(OH)D3, Waters LC-MS/MS device liquid chromatography mass spectrometry was used.

STATISTICAL ANALYSIS

Calculations were performed using SPSS version 10.1. Student's test was used to compare the groups in a parametric way (for data showing homogenous dispersion) and Mann Whitney U test was used in a non-parametric way (for data showing non-homogenous dispersion) in T2DM and control groups. Correlation between all variables in patient and control groups was calculated by Pearson correlation analysis. Data were presented as mean ± SD. A p value of <0.05 was considered as statistically significant.

RESULTS

This study was performed with 101 DM patients. Sixty one of them were female (61%), 40 of them were male (29%). In the control group there were 36 (60%) female and 24 (40%) male normal people.

All the demographic and laboratory findings of the groups were compared and illustrated in Table 1. It was found that BMI, waist circumference, hip circumference, FBG, HbA1c, FI, Homeostasis model assesment-insulin resistance (HOMA-IR), systolic blood pressure, diastolic blood pressure, TG, CRP and 25(OH)D3 levels of the DM patients were significantly high compared to the controls (p <0.01 all). Any difference was not observed in age, LDL-C, HDL-C, Hcy, Ca, P and PTH levels of the patient and control groups.

When we made correlation analysis in diabetic patients weak negative correlations between 25(OH)D3 and BMI (p:<0.001, r:-0.23), HbA1c (p:<0.05, r:-0.21), FI (p:<0.05, r:-0.21), HOMA-IR (p:<0.05, r:-0.20), triglyceride (p:<0.05, r:-0.24), moderate negative correlations between CRP (p:<0.05, r:-0.50), systolic blood pressure (p:<0.05, r:-0.30), diastolic blood pressure (p:<0.05, r:-0.40) were found. Correlation analysis in control group revealed weak negative correlation between 25(OH)D3 and BMI (p:<0.001, r:-0.32).

TABLE 1: Demographic and laboratory findings of the groups.

	Diabetes mellitus N: 101	Control N: 60	p
Age (year)	55.8±7.5	51.9±7.7	NS
BMI (kg/m^2)	29.8± 4.1	27.2±2.56	<0.01
Waist cir. (cm)	97.4±10.6	90.5±6.8	<0.01
Hip Cir.(cm)	105.6±9.5	99.6±10.5	<0.01
FBG (mg/dL)	172.1±68.6	92.2±5.0	<0.01
HbA1c (%)	8.2±2.1	5.4±0.2	<0.01
FI (μU/mL)	13.0±6.4	7.9±4.0	<0.01
HOMA-IR	5.4±3.6	1.7±0.9	<0.01
CRP (mg/dL)	8.4±5.3	3.0±1.7	<0.01
Hcy (μmol/mL)	11.2±6.2	10.5±2.2	NS
SBP (mm Hg)	140.1±10.1	114.0±10.4	<0.01
DBP (mm Hg)	92.6±11.5	81.8±9.1	<0.01
LDL-C (mg/dL)	137.7±38.4	134.8±31.0	NS
HDL-C (mg/dL)	34.8±10.0	29.4±10.4	NS
TG (mg/dL)	202.9±23.5	144.4±50.0	<0.01
Ca (mg/dL)	9.4±0.3	9.3±0.5	NS
P (mg/dL)	3.3±0.4	3.3±0.2	NS
PTH (pg/mL)	53.6±20.3	55.2±18.2	NS
25(OH)D3 (ng/mL)	9.9±7.6	14.3±7.7	<0.01

BMI: Body mass index; Waist cir: Waist circumference; Hip cir: Hip circumference; FBG: Fasting blood glucose; HbA1c: Hemoglobin A1c; FI: Fasting insulin; HOMA-IR: Homeostasis model assesment-insulin resistance; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; LDL-C: Low density lipoprotein cholesterol;, HDL-C: High density lipoprotein cholesterol; TG: Triglyceride; CRP: C-reactive protein; Hcy: Homocysteine; Ca: Calcium; P: Phosphorus; PTH: Parathyroid hormone; 25(OH)D3: 25-hydroxy vitamin D3. Data are presented as mean±SD. NS: nonsignificant.

CONCLUSION

In animal studies, 1,25-dihydroxyvitamin D has been shown to regulate the renin-angiotensin system. Vitamin D receptor null mice or mice with inborn deficiency of the 1α-hydroxylase gene develop high renin hypertension and cardiac hypertrophy.[21] Moreover, vascular endothelial and smooth muscle cells respond to exposure to 1,25-

dihydroxyvitamin D with a "favorable cardioprotective" gene response. This corresponds well with reduced thrombogenesis and increased fibrinolysis as observed in vivo.[21] There were studies revealing an inverse relation between 25OHD and cardiovascular disease.[22]

Significantly low 25(OH)D3 concentration among DM patients were found. Our findings are consistent with previous observations.[23-26] Vitamin D insufficiency has long been suspected as a risk factor for type 127-30 and type 2 diabetes mellitus[31-33] and metabolic syndrome.[34-36] As 25(OH)D3 levels of our controls were also below normal limits, vitamin D supplementation in our population, in the prevention of these disorders must be considered.

Obesity is an established risk factor for vitamin D deficiency.[37] Higher storage in adipose tissue is a plausible explanation for increased rates of deficiency of vitamin D in obese individuals.[38] Hyppönen et al. showed that body size was a strong determinant for 25(OH)D3 with concentrations being suboptimal in most obese patients. In their normal, overweight, obese and severely obese subjects serum 25(OH)D3 levels decreased with increasing BMI.[23] Al-Daghri also determined that waist-hip circumference, BMI were significant predictors of 25(OH)D3.26 In a study performed with an Australian inadequate Vitamin D status population, an incresed risk of obesity and DM was found.[39] Barchetta et al. when classified their patients according to serum 25(OH)D3 quartiles, found increasing BMI and waist circumference results, in lowest vitamin D quartiles to increasing.[40] In our study, where 25(OH)D3 levels were significantly low compared to controls, waist and hip circumference and BMI levels were high in consistence with previous studies. There was also negative correlation between 25(OH)D3 levels and BMI of DM patients. Negative correlation was also found between 25(OH)D3 levels and BMI of controls. We think that our results strenghten the opinion about the relation of obesity and low VD levels.

In our study negative correlation was also demonstrated between HbA1c and 25(OH)D3 levels in diabetic patients. In Hyppönen's study the decrease in HbA1c was correlated with the increase in 25(OH)D3 in Caucasian subjects.[23] In studies with DM patients, vitamin D deficiency was found to be independently related to HbA1c in diabetic women.[25,41] In a Polish study low 25(OH)D3 levels were found to be associated with higher blood glucose levels in obese adolescents.[38]

As there were authors examining DM, who found no relation between insulin resistance and low vitamin D levels,[42] there were other ones who showed a positive correlation of 25(OH)D3 concentration with insulin sensitivity.[3,42-44] In the Polish study just mentioned, low 25(OH)D3 levels were also found to be associated with higher insulin and HOMA-IR in obese patients.[38] A 10 year prospective study indentified an inverse relationship between baseline serum 25(OH)D3 concentrations and later risk of insulin resistance.[45] Administration of supplemental vitamin D to insulin resistant subjects has resulted in an improvement in insulin sensitivity.[46,47] In concordance with most of the recent studies our diabetic patients besides having low levels of 25(OH)D3 levels had high HOMA-IR levels. There was also negative correlation between vitamin D and HOMA-IR levels of the patient group. As we did not have the chance of following up insulin resistant patients after supplementation of vitamin D, we could not be able to show the effect of Vitamin D in our patients. While optimal Vitamin D concentrations for reducing insulin resistance were shown to be 80-110nmol/L (32-44ng/mL), it is evident that too much effort must be really expanded.[46]

There have been at least two mechanisms postulated for an increase in insulin sensitivity in response to improved vitamin D status-suppression of chronic inflammation and increased expression of the insulin receptor and/or proteins of insulin signalling cascade. A mild inflammatory state, marked by the presence of proinflammatory cytokines, such as tumor necrosis factor- α (TNF-α) and interleukin-6 (IL-6) associated with insulin resistance.[48] Vitamin D has been shown to dose dependently suppress the release of TNF-α and IL-6.[49] CRP besides being a cardiovascular risk factor, was considered to be a useful biomarker for the pres-

ence of TNF-α and IL-6, In the present study we measured only high sensitivity CRP. Consistent with previous studies that found a relation with vitamin D and CRP, in our study CRP levels of the diabetics, whose 25(OH)D3 levels were low, were significantly higher than the controls.[50,51] There was a negative correlation between 25(OH)D3 and CRP. Although negative correlation between 25(OH)D3 and BMI was also found in our control subjects, our DM patients were obese, having higher BMI and wasit-hip circumferences. Inflammatory effects of increased adiposity, as being a cause of insulin resistance, must be kept in mind. The anti-inflammatory action of vitamin D may also explain increased insulin resistance. In future studies, measuring TNF-α and IL-6 and perhaps also metalloproteinases, which are inflammatory markers and are associated with vascular damage may enlighten probable interactions of these inflammatory markers, vitamin D and insulin resistance.

Plasma homocysteine (Hcy) level is also considered to be a marker of endothelial dysfunction and shown to be a predictor of cardiovascular disease in epidemiological studies.[52,53] Keeping in mind its probable role in cardiovascular abnormalities we wanted to evaluate Hcy levels in our diabetics and control. There was not statistically significant difference in the levels of DM patients and control, we did not find any correlation between 25(OH)D3 and Hcy levels in diabetics. Bonakdaran et al. also stated that CRP levels was higher, but not Hcy, in their vitamin D deficient DM patients, compared to controls.[42] In the literature, there were similar study results, examining cardiovascular risk factors and vitamin D which found no relation with vitamin D and Hcy.[54,55]

Increasing evidence indicates that vitamin D may influence hypertension, which is another risk factor for cardiovascular disease. Bhandari et al. in their study found hypertension rates as 20%, 27%, 41%, and 52%, in 25(OH)D3 quartiles ≥40ng/mL, 30-39ng/mL, 15-29ng/mL and < 15 ng/mL respectively.[56] A high prevalence of vitamin D deficiency was demonstrated in hypertensive pregnant women.[57] In a meta-analysis, where 18 studies were included, blood 25(OH)D3 concentrations were found to be associated with hypertension.[58] Vitamin D seems to have an inhibitory effect on renin secretion.[59] It was also suggested that vitamin D improves endothelial function, blunts cardiomyocyte hypertrophy, improves insulin sensitivity, reduces concentrations of serum free fatty acids and regulates the expression of the natriuretic peptide receptor.[60] In the correlation analysis of our diabetic patients, systolic and diastolic pressure were found to be negatively correlated with 25(OH)D3 levels. Comparing our DM patients with controls both systolic and diastolic blood pressures were higher than the controls, making us think about the relation of low vitamin D levels with hypertension.

Low serum levels of 25(OH)D3 have been associated with an unfavorable lipid profile, which could possibly explain the relation with cardiovascular disease and mortality. In all cross-sectional studies low levels of vitamin D was found to be associated with low HDL-C, resulting in a favorable LDL-C to HDL-C ratio.[61,62] There is also a uniform agrement between studies on a negative relation between serum 25(OH)D3 and TG.[62,63] On the other hand, the intervention studies gave divergent results, with some showing a positive and some a negative effect of vitamin D supplementation.[64-66] We did not find any difference HDL-C and LDL-C levels in our diabetics and controls, and any correlation between those lipids and vitamin D. The lack of the difference may be explained by high LDL-C and low HDL-C levels we demonstrated also in our controls. However we showed negative correlation in 25(OH)D3 and TG levels, and higher TG levels in diabetics compared to controls. As the effect of vitamin D supplementation on serum lipids is at present uncertain, we are waiting for the results of forthcoming vitamin D intervention studies before drawing a conclusion on potential effects of vitamin D on lipid profiles.

Obesity results in morbidity and mortality largely because of its association with other diseases including diabetes and cardiovascular disease. The relation with obesity and insulin resistance-diabetes was now a well known phenomenon. Our diabetic patients were more obese as shown in their

BMI's and waist and hip ratios. When evaluating the metabolic values of diabetics we think that changes about obesity must also be considered.

We think that examining our groups in winter season is a real limitation of our study. This result is evident in low 25(OH)D3 levels in both control and patient groups. We planned to perform another study in other seasons and with larger groups.

In conclusion, our study demonstrated that vitamin D levels were low in type 2 diabetic patients. As there is an inverse association between vitamin D and obesity-insulin resistance-glucose metabolism indices, blood pressure, triglyceride, C-reactive protein and as 25(OH)D3 levels of normal subjects were also under limits, we think that vitamin D supplementation is required in our population.

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