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Effect of Vitamin D Treatment on Glycemic Control, Diastolic Functions, and Carotid Intima-Media Thickness in Patients with Type 2 Diabetes Mellitus

Tip 2 Diyabetes Mellitus Hastalarında Vitamin D Replasmanının Glisemik Kontrol, Diyastolik Fonksiyon ve Karotis İntima-Media Kalınlığı Üzerine Etkisi

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Abstract

Objective: The purpose of this study was to investigate the influence of 25(OH)D3 levels on glycemic control, diastolic functions, and carotid intima-media thickness in patients with Type 2 diabetes mellitus.

Material and Methods: Patients admitted to the endocrinology department, with the diagnosis of Type 2 diabetes mellitus, who were under follow-up for at least six months and also had 25(OH)D3 deficiency [25(OH)D3 levels <20 ng/mL] were included in this study. D3 supplement (50.000 IU) was administered to the patients every month up to six months. Carotid intima-media thickness was measured using the B-mode ultrasonography. Diastolic function was evaluated using the tissue doppler imaging by measuring tissue e wave/tissue a wave (e'/a') and annular E wave/tissue e wave (E/e') ratios. All the evaluations were made at baseline and at six months after vitamin D3 supplementation.

Results: A total of 45 (27 females, 18 males; mean age: 56.2±7.8 years) patients were included in this study. The mean duration of diabetes was 8.5±6.8 (ranging from 7.0 to 9.0 years) years. It was found that even after D3 supplementation, fasting plasma glucose and Hemoglobin A1C levels did not change, yet, the carotid intima-media thickness reduced (788±100 μm vs. 745±116.8 μm; p=0.009). Diastolic function parameters e'/a' (0.79±0.21 vs. 0.89±0.26; p=0.03) and E/e' (7.27±1.81 vs. 6.52±1.65; p=0.048) also improved significantly after the therapy.

Conclusion: Vitamin D supplementation, in patients with Type 2 diabetes mellitus, who are also having vitamin D deficiency, seems to be beneficial in reducing the thickness of carotid intima-media, which is a well-known cardiovascular risk predictor, and in improving diastolic functions by vitamin D repletion. Further prospective well-designed studies with a larger patient population are needed to lead a firm conclusion in this regard.

Keywords: Type 2 diabetes mellitus; 25(OH)D3; glycemic control; carotid intima-media thickness; diastolic function; vitamin D deficiency

Özet

Amaç: Çalışmamızda Tip 2 diabetes mellitus tanısı ile takip ettiğimiz hastalarda 25(OH)D3 düzeyleri ve bunun glisemik kontrol, diyastolik fonksiyon ve karotis intima-media kalınlığı üzerine etkilerinin incelenmesi amaçlanmıştır.

Gereç ve Yöntemler: Çalışmaya endokrinoloji bölümüne başvuran ve en az altı ay boyunca Tip 2 diabetes mellitus tanısı ile takip edilen ve 25(OH)D3 eksikliği [25(OH)D3 seviyesi <20 ng/mL] olan hastalar dâhil edildi. Hastalara altı ay boyunca aylık D3 desteği (50.000 IU) uygulandı. Karotis intima media kalınlığı B-mod ultrasonografi ile ölçüldü. Diyastolik fonksiyon doku e dalgası/doku a dalgası (e'/a') ve anüler E dalgası/doku e dalgası (E/e') oranları ölçülerek doku Doppler görüntüleme ile değerlendirildi. Tüm değerlendirmeler başlangıçta ve vitamin D3 takviyesinden altı ay sonra yapıldı.

Bulgular: Çalışmamıza toplam 45 (27 kadın, 18 erkek; ortalama yaş: 56.2±7.8 yıl) hasta alındı. Ortalama diyabet süresi 8.5±6.8 yıl'dı (7.0-9.0). Vitamin D3 takviyesi sonrası açlık plazma glukozu ve Hemoglobin A1C düzeyleri değişmedi, ancak karotis intima-media kalınlığı azaldı (788±100 μm vs 745±16,8 μm; p=0,009). Diyastolik fonksiyon parametrelerinde e'/a' (0,79±0,21'e karşı 0,89±0,26; p=0,03) ve E/e' (7,27±1,81'e karşı 6,52±1,65; p=0,048) tedaviden sonra önemli ölçüde iyileşme görüldü.

Sonuç: Vitamin D eksikliği olan Tip 2 diabetes mellituslu hastalarda Vitamin D takviyesi, iyi bilinen bir kardiyovasküler risk prediktörü olan karotis intima-media kalınlığının azaltılmasında ve diyastolik fonksiyonların iyileştirilmesinde yararlı görünmektedir. Daha kesin sonuçlar için daha büyük hasta populasyonu ile daha iyi tasarlanmış prospektif çalışmalar gereklidir.

Anahtar kelimeler: Tip 2 diabetes mellitus; 25(OH)D3; glisemik kontrol; karotis intima media kalınlığı; diyastolik fonksiyon; vitamin D eksikliği

The summary of the study presented as an oral presentation and was published in the 52th National Diabetes Congress, 13-18 November 2018, Antalya, Abstract Book (abstract <250 words).

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Introduction

Vitamin D is a steroid prohormone, synthesized when the skin is exposed to ultraviolet (UVB) light. It can also be obtained through dietary intake or supplementation (1). Vitamin D exists in two forms: Vitamin D2 (ergocalciferol) and D3 (cholecalciferol). Vitamin D (D2 and D3) is then subsequently hydroxylated in the liver by the enzyme 25-hydroxylase to produce 25 hydroxyvitamin D [25(OH)D] (2, 3). 25(OH)D is then further hydroxylated in the kidneys to form 1,25(OH)2D3, which is the biologically active form of vitamin D. The serum level of 25(OH)D with a half-life of three weeks represent the principal index of vitamin D status (4, 5). The serum concentration of 25(OH)D below 20 ng/mL is considered as vitamin D deficiency and the treatment target is taken as 25(OH)D levels >30 ng/mL. Daily supplementation of 1000 IU (maximum 4000 IU/day) of D2 or D3 is advised to reach the target values (5, 6).

In the recent years, vitamin D has been found to have a protective role against immune dysfunction, myopathy, cancer, hypertension (HT), cardiovascular disorders, insulin resistance, metabolic syndrome (MS), and T2DM (5, 7, 8). The gene for VDR was discovered in 1988 and found to be present in the cells of many tissues, including parathyroid cells, pancreatic cells, macrophages, keratinocytes, special nerve cells, and renal tubular cells. It was observed that vitamin D receptor regulates approximately 3% of the human genes via its endocrine effect (6). These mechanisms of endocrine effects that improve insulin resistance, along with suppressed levels of pro-inflammatory cytokines, may provide an explanation for the relationship between glucose metabolism and vitamin D (7, 9, 10). Earlier studies have shown that vitamin D deficiency is associated with increased PTH levels and the risk of developing HT and cardiovascular disorders, due to the effects of vitamin D on parathormone (PTH) release and its direct effects on renin-angiotensin-aldosterone (RAA) system and cardiac muscles (5, 11, 12). In addition, a relationship between vitamin D deficiency and diabetic neuropathy has also been observed (13, 14). An increase in the carotid intima-media thickness is considered to be a risk factor for coronary atherosclerosis (15). Serum 25(OH)D levels have been found to be associated with an increase in carotid intima-media thickness at baseline and progression of carotid intima-media thickness is related to other factors of cardiovascular risks, such as lipoprotein levels (16). Epidemiological studies have also shown that vitamin D support therapy allows better glycemic control in T2DM patients and reduces the risk of cardiovascular disorders (7, 17, 18). However, the studies on the effect of Vitamin D3 support for neuropathic pain and early risk predictors for cardiovascular disorders in patients with T2DM having 25(OH)D deficiency are limited in number.

The authors aim to investigate the effect of vitamin D3 support on insulin resistance, glycemic control, neuropathic pain, and cardiovascular risk predictors in patients with T2DM.

Material and Methods

Inclusion and Exclusion Criteria

This study was conducted at Cukurova University, Faculty of Medicine between January 2014 and November 2015 after being approved by the (number of meetings: 28, February 14, 2014). Patients admitted to the endocrinology department, with a diagnosis of T2DM, having 25(OH)D deficiency in their blood [25(OH)D3 levels <20 ng/mL] were included in this study. Written informed consent was obtained from all patients. The patients with chronic liver diseases, chronic renal failure, decompensated heart failure, secondary osteoporosis, metabolic bone disease, malignancy, and those who received Ca and vitamin D supplement within the past three months and with coronary artery disease were excluded from the study. The study was performed in accordance with the principles of the Declaration of Helsinki.

Characteristics of the Patients and Management

Age, sex, duration of T2DM, smoking habits and medication history, chronic complications of diabetes, and comorbid conditions were recorded. The patients were made to attend control visits at six-month intervals, during which a physical examination was conducted, which also included measurements for body mass index (BMI) and sys-

tolic and diastolic blood pressures. The patients were assessed for the development of diabetic neuropathy using the LANSS-pain scale. The patients with a LANSS-pain scale score of more than 12 points were considered to be having diabetic neuropathy. Vitamin D supplementation was administered to the patients for six months at a dose of 50,000 IU/month orally.

Laboratory Tests

Complete blood count, fasting plasma glucose (FPG), HbA1c, insulin, calcium (Ca), inorganic phosphor (P), low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglyceride levels were measured at baseline and after six months. In addition, alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate transaminase (AST) levels were measured to rule out liver diseases affecting vitamin D metabolism and non-alcoholic fatty liver disease. Also, Lipoprotein (a) (normal: <300 mg/L), vitamin B12 (normal range: 126.5-505 pg/mL), PTH (normal range: 12-88 pg/mL), and 25(OH)D3 (normal range: 10-60 ng/mL) were measured.

Insulin resistance of the patients was assessed by the hemostatic model assessment-insulin resistance (HOMA-IR) method and the patients with values ≥2.24 were considered to be insulin resistant: HOMA-IR: FPG (mmol/L)×Insulin (μIU/mL)/22.5 (13). Serum glucose, HDL, LDL, triglyceride, lipoprotein (a), Ca, and P levels were measured using a Beckman Coulter DXC 800 device and the photometric method. HbA1c was measured using the immunoturbidimetric method. 25(OH)D3 levels were measured using HPLC (Bio-Rad Laboratories, München, Germany), while insulin, TSH, and PTH levels were determined using a Beckman Coulter DXI 800 device and the chemiluminescence method.

Echocardiographic Assessment

The patients underwent B-mode transthoracic echocardiography and tissue Doppler imaging at baseline and six months later. B-mode echocardiography was primarily performed to rule out any presence of structural heart disease. The measurements of mitral annular early diastolic wave/tissue Doppler early diastolic wave ratio (E/e') and tissue Doppler early diastolic wave/atrial contraction wave ratio (e'/a') were performed in all the patients. Further, the myocardial performance index (MPI) was calculated in the TDI recordings. In accordance with recommendations from the American Echocardiography Association, all the procedures were carried out by a single operator, in the middle of the day, in order to eliminate the effects of circadian changes on diastolic dysfunction.

The patients were asked to lie down in a horizontal position on their backs for the measurement of carotid intima-media thickness with their heads tilted backward. A single operator carried out the examination of the left and right carotid arteries. The patients were initially subjected to a general morphological evaluation of both common carotid arteries (CCA) and cervical segments of the internal carotid artery (ICA), following the differentiation of the carotid artery under axial and longitudinal plane through B mode grayscale imaging. Carotid intima-media thickness was measured in the region near the carotid bifurcation. Measurements were made both, in the left and right CCAs and mean values were calculated.

Statistical Analysis

Statistical analysis was performed using the IBM SPSS version 20.0 software (SPSS Inc., Chicago, IL, USA). The categorical variables were expressed as numbers and percentages, whereas continuous variables were summarized as mean with standard deviation, median, and minimum-maximum, wherever appropriate. The normality of distribution for continuous variables was analyzed using the Kolmogorov-Smirnov test. For comparison of before-after measurements, paired sample t-test or the Wilcoxon Signed Rank test was applied. To evaluate the correlations between measurements, Pearson's Correlation Coefficient or Spearman's Rank Correlation Coefficient was employed.

Results

The study included 45 patients (27 females, 18 males) with the diagnosis of T2DM who underwent follow-up for at least six months. The mean age of the patients was 56.2±7.8 years (37-69). Demographic features of the patients, the complications and comorbidi-

ties are presented in Table 1. Among the patients, 87% were on oral anti-diabetic medications. Hypertension was the most frequent comorbidity (46%). The LANSS-pain score was found to be ≥12 points in 12 patients (32%) and these patients were considered to have neuropathic pain.

Clinical response of the patients to vitamin D3 supplementation therapy is summarized in Table 2. A significant increase in 25(OH)D3, Ca, and P levels was observed (p=0.00, p=0.01, p=0.04, respectively). However, the PTH levels were found to decrease significantly (p=0.025). Although there was a reduction in LANSS-pain score (7.1±6.2 vs. 5.8±5.7; p=0.128), the change was not statistically significant.

The changes in carotid intima-media thickness and cardiac functions are listed in Table 3. Carotid intima-media thickness of the patients was significantly decreased after vitamin D supplementation (788±100 μm vs. 745±116.8 μm; p=0.009). Mitral E/e' ratio was decreased (7.27±1.81 vs. 6.52±1.65; p=0.048) and tissue e'/a' ratio was increased (0.79±0.21 vs. 0.89±0.26; p=0.03) significantly after vitamin D therapy. However, no significant change was observed in MPI values even after the therapy.

Table 1. Demographic and clinical features of the patients.

	Mean±SD
Age (years)	56.2±7.8
Duration of diabetes (years)	8.5±6.8
Body Mass Index (BMI) (kg/m²)	30.9±4.6
Systolic Blood Pressure (mmHg)	136±17.6
Diastolic Blood Pressure (mmHg)	69.8±27
HbA1c (%)	6.6±0.4
Oral antidiabetic (OAD) users N, (%)	39 (87%)
OAD + Insulin users N, (%)	5 (11%)
Only insulin users N, (%)	1 (2%)
Smokers N, (%)	8 (17.8%)
Diabetic nephropathy N, (%)	1 (2.2%)
Diabetic retinopathy N, (%)	1 (2.2%)
Painful diabetic neuropathy N, (%)	12 (32%)
Hypertension N, (%)	13 (28.9%)
Hypertension+Hyperlipidemia N, (%)	8 (17.8%)

Discussion

In this study, patients with T2DM who were deficient in 25(OH)D3 were treated with vitamin D therapy. After six months of vitamin D supplementation, a significant decrease in carotid intima-media thickness measurements along with significant improvements

Table 2. The biochemical parameters of the patients at baseline and six months (Mean±SD).

	Baseline	6 Months	P value
Hemoglobin (g/dL)	13.1±1	13.1±1.1	0.6
Glucose (mg/dL)	128±33	127±26	0.77
HbA1c (%)	6.6±0.4	6.6±0.7	0.9
HOMA-IR	2.9±2.5	2.4±1.5	0.23**
Creatinine (mg/dL)	0.66±0.1	0.7±0.2	0.03*
ALT (U/L)	24±11	22±11	0.24
Calcium (mg/dL)	9.3±0.46	9.5±0.5	0.01*
Phosphorus (mg/dL)	3.7±0.6	3.9±0.5	0.04*
HDL-C (mg/dL)	46±9	45±9	0.16
LDL-C (mg/dL)	122±45	112±42	0.15
Triglycerides (mg/dL)	153±73	152±77	0.9
Lipoprotein (a) (mg/dL)	249±306	229±319	0.26#
PTH (pg/mL)	48±24	41±18	0.025*
25 OH Vit D3 (ng/mL)	16.7±10.6	40.5±18	0.000*
LANSS-pain score	7.1±6.2	5.8±5.7	0.128£

* p<0.05
** Baseline: Median: 2 (min: 0.86-max: 16.7); six months: Median: 1.95 (min: 0.90-max: 7).
# Baseline: Median: 109 (min: 0.7-max: 1366); six months: Median: 105 (min: 5.4-max: 1651).
£ Baseline: Median: 7 (min: 0-max: 21), six months: Median: 5 (min: 0-max: 266).

Table 3. Echocardiography, Doppler and carotid intima-media thickness measurements at the baseline and at six months follow-up after correcting vitamin D levels.**

	Baseline	6 Months	P value
EF (%)	60.5±1.8	60±0.79	0.163
IVS (mm)	9.8±1.3	10.2±1.23	**0.038***
LA (mm)	32.6±3.5	33±2.7	0.17
E (cm/sec)	66.3±18	68.2±16.4	0.258
A (cm/sec)	75.4±16.8	75.4±19	0.97
CIMT (μm)	788±100	745±116.8	**0.009***
Tissue e/Tissue a	0.79±0.21	0.89±0.26	**0.03***
Annular E/Tissue e	7.27±1.81	6.52±1.65	**0.048***
MPI left	0.36±0.06	0.36±0.06	0.744

* p<0.05.
** EF: Ejection Fraction, E: Peak myocardial early diastolic flow rate, A: Peak myocardial late diastolic flow rate, IVS: Interventricular septum, CIMT: Carotid Intima Media Thickness, LA: Left Atrium, MPI: Myocardial Performance Index.

in diastolic functions of the patients was observed.

Vitamin D deficiency is a common condition and some studies have established the relationship between vitamin D deficiency and insulin resistance, T2DM, and cardiovascular disorders (5, 7, 19).

This study aimed to evaluate the effects of vitamin D supplementation on glycemic control, diabetic neuropathy, diastolic dysfunction, and carotid intima-media thickness (one of the indicators of cardiovascular risk), in patients with T2DM and low serum levels of 25(OH)D3.

In several studies, it has been suggested that vitamin D plays a functional role in glucose tolerance through its effects on insulin secretion and insulin sensitivity. Animal studies have proved that vitamin D is a fundamental factor for normal insulin secretion. Probably, vitamin D decreases insulin resistance through its effects on both, regulation of the insulin receptor gene and, Ca and P metabolism (7, 9, 10). Some mechanisms are related to the effect of vitamin D, such as the presence of VDR on pancreatic β cells since vitamin D activating 1α hydroxylase is expressed in pancreatic β cells, the presence of VDR in skeletal muscles, and the fact that 1,25(OH)2D increases transcription of insulin receptor genes (12, 13). The studies conducted on healthy subjects have shown that decreased vitamin D levels cause beta cell dysfunction (20-22). Some studies showed that serum 25(OH)D concentrations were significantly lower in patients with T2DM as compared to that in healthy controls. Furthermore, vitamin D supplementation in 5,677 patients with impaired glucose tolerance was found to have increased insulin sensitivity in 54% of the patients (12, 23, 24).

In another study, 300 patients with T2DM, who underwent lifestyle changes and were administered metformin, sulfonylurea, or a combination of these medications, received vitamin D in a dose of 50,000 IU monthly or a placebo for six months. This study found that at least a six-month follow-up period was required to observe a significant change in HbA1c values and the patients receiving vitamin D supplementation achieved a better glycemic control (7). In the present study, no significant change was observed in glycemic levels of the patients at six months; though, there was an insignificant decrease in HOMA-IR levels. Despite significant increase in the serum 25(OH)D3 concentrations in the patients at the end of the study, the lack of any significant change in glycemic parameters may be attributed either to near-normal HbA1c values in patients at the baseline, or the duration of six-month follow-up was insufficient to evaluate any change in the HbA1c values, or other individual factors of the patients. Vitamin D was used in the different dosages and durations in previous studies (7, 25, 26). Talaei et al. administered oral vitamin D3 50,000 IU weekly to 100 patients with T2DM for eight weeks and, in contrast to the findings of the present study, they showed significant decreases in FPG, insulin, and HOMA-IR values (12).

The current International Osteoporosis Foundation Guidelines recommend serum 25(OH)D level of 30 ng/mL (75 nmol/L) for maximum PTH suppression (27). In the present study, 25(OH)D levels reached above 30 ng/mL at the sixth month; and in consistency with previous studies, PTH levels showed a decrease with vitamin D supplementation.

Clinical studies have shown that vitamin D exerts direct influence on cardiomyocytes and indirect influences on the cardiovascular system through its effects on the circulating hormones and calcium levels (28). In addition, it has been shown that vitamin D levels are associated with hypertension and cardiovascular endpoints and that vitamin D supplementation decreased the risk of cardiovascular events (29-31). Wang et al. evaluated 25(OH)D concentrations in 1,700 patients with no cardiovascular disease and showed that levels below 37 nmol/L increased the incidence of cardiovascular events 1.62 times (32). The increased risk was associated with possible effects of low vitamin D levels, effects of the RAA system with inhibition of renin expression and impairment in vascular functions, such as inflammation, smooth muscle hypertrophy, and thrombosis. Some studies have shown a significant relationship between carotid intima-media thickness and intima-media thickness progression and serum 25(OH)D concentration, which are important indicators of atherosclerosis and coronary artery atherosclerosis (24, 33). Furthermore, hypovitaminosis D has been observed to be an independent risk factor for increased carotid intima-media thickness, and vitamin D supplementation has been found to be associated with improvement in carotid intima-media thickness in patients with T2DM, as compared to non-diabetic patients (16, 34, 35).

In this study, a significant decrease in carotid intima-media thickness measurements in patients was observed after vitamin D3 supplementation. On the other hand, vitamin D supplementation may not be the only cause of improvement in carotid intima-media thickness; instead, improvement in other metabolic parameters may also have contributed to this progress. Significant improvements in Ca and PTH levels and minimal improvements in HOMA-IR and lipoprotein (a) levels may have favorably affected cardiac functions. Although the metabolic benefits and cardio-protective effects of vitamin D are well known, it could not be made clear whether vitamin D deficiency and serum 25(OH)D levels were associated with cardiovascular morbidity and mortality.

Study Limitations

The major limitation of this study was its limited patient population and relatively short term follow-up period. Moreover, the lack of investigations on cardiovascular endpoints is another deficit. The fact that the present study is not a placebo/controlled study is another important limitation.

Conclusion

In conclusion, the most important results observed in this study are the improvement in cardiac diastolic functions and decrease in carotid intima-media thickness after vitamin D supplementation in patients with uncomplicated T2DM. Although, no improvements in the metabolic indices associated with glycemic control were observed. However, well-controlled studies with a larger patient population, specifically designed to evaluate cardiovascular mortality and morbidity are warranted to make a firm conclusion in this regard.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Ethical statement

All procedures performed in studies involving human participants were in accordance

with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Authors Contributions

Idea/ Concept: Mehtap Evran Olgun, Tamer Tetiker; Design: Mehtap Evran Olgun, Tamer Tetiker; Data collecting: Mehtap Evran Olgun, Gamze Akkuş; Echocardiography: Mustafa Gök, Çağlar Emre Çağlıyan; Statistical analysis: İlker Ünal; Writing: Mehtap Evran Olgun, Gamze Akkuş; Editing: Çağlar Emre Çağlıyan, Tamer Tetiker, Murat Sert;Review and final approval: Mehtap Evran Olgun, Gamze Akkuş, Mustafa Gök, Çağlar Emre Çağlıyan, İlker Ünal, Murat Sert, Tamer Tetiker.

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