

Research Article

Association between Vitamin D Level and Microvascular Complications in Patients with Type 2 Diabetes

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Abstract

Objectives: The aim of this study was to further investigate the prevalence of Vitamin D deficiency in diabetes mellitus (DM) patients, as well as the association between hypovitaminosis D and the microvascular complications of type 2 (T2) DM.

Methods: This was a cross-sectional, case-control study of 206 T2DM patients and 34 healthy control subjects. Participants were evaluated for the presence of diabetic retinopathy, nephropathy, and neuropathy.

Results: The level of serum 25-hydroxyvitamin D (25(OH) D) was significantly lower in the T2DM patients (11.16 ± 3.99 ng/mL vs. 15.58 ± 3.16 ng/mL; $p < 0.05$). Furthermore, 2.7% of the patients were found to have retinopathy ($n=53$), 3.6% had neuropathy ($n=65$), and 29.1% of the patients had microalbuminuria ($n=60$). Although serum 25(OH) D levels were significantly lower in the presence of retinopathy and neuropathy ($p < 0.05$ for both), no significant association between Vitamin D level and microalbuminuria was found.

Conclusion: An inverse relationship between the circulating 25(OH) D level and the prevalence of retinopathy and neuropathy in T2DM patients was determined. However, there was no significant association between microalbuminuria and the 25(OH) D level.

Keywords: Microvascular complications, type 2 diabetes, vitamin D deficiency

Vitamin D is absorbed into the body through exposure to sunlight, a diet of vitamin-fortified foods, and dietary supplements. The major source of vitamin D absorption is through skin synthesis. Vitamin D receptors have been found in various tissues, including the brain, prostate, breast, colon, pancreas, and immune cells.^[1] The estimated worldwide prevalence of vitamin D (25-hydroxyvitamin D [25(OH) D]) deficiency is 30% to 87%.^[2] A study in Turkey in 2000 demonstrated that the deficiency prevalence ranged

between 44% and 100% in women.^[3] Furthermore, studies have revealed a high prevalence of vitamin D deficiency in type 2 diabetes mellitus (T2DM) patients in the Turkish population.^[4, 5] Recently, vitamin D was found to have various effects on glucose metabolism, obesity, metabolic syndrome, cardiovascular diseases, and cancer, as well as bone metabolism. It has been suggested that vitamin D is involved in insulin sensitivity by controlling calcium flux through the membrane in both beta-cells and peripheral

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insulin-target tissues.^[6]

Diabetic microvascular complications are clinically reflected in diabetic nephropathy (DN), diabetic retinopathy (DR), and diabetic peripheral neuropathy (DPN). Different mechanisms have been proposed in various studies, including increased formation of advanced glycation of end products, polyol pathways, protein kinase C pathways, and oxidative stress.^[7] Further studies have suggested that low vitamin D levels are related to insulin resistance^[8], higher fasting serum glucose concentrations^[9], and higher levels of glycated hemoglobin (HbA1c).^[10,11] However, few studies have focused on the link between vitamin D and microvascular complications. The results of an observational study by Suzuki et al.^[12] demonstrated that diabetic retinopathy is significantly associated with a low vitamin D level, and that serum 25(OH) D level decreases according to the number of diabetic microvascular complications. Given the limited available data on the relationship of vitamin D insufficiency to microvascular complications, the aim of this study was to examine serum 25(OH) D concentrations as well as DN, DR, and DPN in T2DM patients.

Methods

Study Population

Between January and June 2014, 206 T2DM patients and 34 healthy participants were enrolled in this study. The presence of DM was determined by: a previous diagnosis of T2DM; a random plasma glucose level of 200 mg/dL or greater; the classical features of DM, such as polyuria, polydipsia, polyphagia, and weight loss; a fasting blood glucose level of 126 mg/dL or greater; or an HbA1c level of 6.5% or greater.

Exclusion criteria included any of the following conditions: diagnosis of impaired fasting glucose, glucose intolerance and type 1 DM, presence of macrovascular complications, individuals under replacement therapy for osteoporosis or osteopenia that included both vitamin D and calcium supplementation, diagnosis of primary hyperparathyroidism, or corticosteroid therapy for any reason. Any patients with overt chronic renal failure were also excluded. The study protocol was approved by the Bakirkoy Sadi Konuk Research and Education Hospital Ethics Committee, Istanbul.

Measurements

Hypertension was defined as use of antihypertensive drug therapy, or a systolic blood pressure ≥ 140 mmHg, and/or diastolic blood pressure ≥ 90 mmHg. Body mass index (BMI) was obtained using the formula of weight (kg)/height² (m). Obesity was defined as a BMI of >30 kg/m². Waist circumference was measured at the level of the umbilicus in a stand-

ing position. Fasting plasma samples were obtained from the patients and the control group.

Serum cholesterol, triglyceride, high-density lipoprotein cholesterol, albumin, parathormone, calcium, and phosphorus were measured using enzymatic colorimetric methods with commercially available kits (Cobas C311; Roche Diagnostics GmbH, Mannheim, Germany), and low-density lipoprotein cholesterol C was calculated according to the Friedewald formula. Serum glucose measurements were determined enzymatically using the hexokinase method (PRODUCT?; Roche Diagnostics GmbH, Mannheim, Germany). Blood HbA1c was determined with a Cobas C311 analyzer using the particle-enhanced immunoturbidimetric method (PRODUCT?; Roche Diagnostics, Mannheim, Germany).

Final results were expressed as percent of HbA1c of the total hemoglobin according to the protocol of the Diabetes Control and Complications Trial/National Glycohemoglobin Standardization Program. A Behring BN-100 nephelometer (Behring Diagnostic, Frankfurt, Germany) using the particle-enhanced immunoturbidimetric method was employed to measure C-reactive protein (CRP). Serum 25(OH) D was measured with an enzyme immunoassay kit in a single laboratory using the same lab assay.

According to guidelines, vitamin D status was estimated by measuring serum 25(OH) D. The definition of vitamin D deficiency was a 25(OH) D level of less than 20 ng/mL, and a 25(OH) D level of 21 to 29 ng/mL was considered to be an insufficiency of vitamin D, according to the World Health Organization definition. Glomerular filtration rate (GFR) was estimated with a 24-hour urine creatinine clearance. Microalbuminuria was investigated in 24-hour-urine samples, and if the urine albumin level was less than 30 mg/24 hours, the patient was defined as normoalbuminuric. Values between 30 and 300 mg/24 hours were indicative of microalbuminuria, and a value greater than 300 mg/24 hours revealed macroalbuminuria. The presence of abnormal albuminuria was confirmed in at least 2 of 3 consecutive samples.

DN was defined as the presence of abnormal albuminuria (microalbuminuria and macroalbuminuria) and/or e-GFRMDRD <60 mL/minute/1.73 m². All subjects were examined for DR by the same ophthalmologist. DR was defined as the presence of at least 1 micro aneurysm or hemorrhage, or exudates in either eye.^[13] Subjects were asked about any history of numbness, paresthesia, tingling sensations, burning sensations and, in the presence of any complaint, patients were referred to the neurology department. Patients diagnosed with diabetic neuropathy were noted.

Table 1. Clinical and biochemical characteristics of the patients and controls

	Patient group (n=206) Mean±SD./n, (%)	Control group (n=34) Mean±SD./n, (%)
Age (years)	55.67±10.43	54.82±10.63
Gender		
Male	81 ±39.3	17±50.0
Female	125±60.7	17±50.0
BMI (kg/m ²)	32.03±6.22	27.34±3.99**
Waist circumference (cm)	105.03±14.21	94.71±9.80**
Systolic pressure (mmHg)	130.79±20.45	118.53±8.40**
Diastolic pressure (mmHg)	75.34±11.94	73.97±7.86
25-OH vitamin D (ng/mL)	11.16±3.99	15.58±3.16*
Fasting blood glucose (mg/dL)	186.75±81.66	85.47±11.34**
HbA1c (%)	8.70±2.28	5.43±0.51**
Triglyceride (mg/dL)	162.52±1.64	141.41±1.56
Total cholesterol (mg/dL)	207.69±48.76	213.62±36.36
LDL (mg/dL)	125.29±45.74	127.94±31.71
HDL (mg/dL)	48.32±11.51	48.47±13.05
CRP (mg/L)	5.05±2.03	2.29±1.83**
Calcium (mg/dL)	9.29±0.48	9.24±0.59
Phosphorus (mg/dL)	3.45±0.54	3.34±0.51
Albumin (g/dL)	4.30±0.33	4.21±0.46
Parathyroid hormone (pg/mL)	51.75±1.49	43.44±1.46*
Creatine clearance (mL/min)	121.13±49.82	162.00±81.03**
Oral antidiabetic drug	125±60.1	0
Oral antidiabetic plus insulin	49±23.8	0
Insulin	163±6.3	0
Antihypertension drug	91±44.2	0
Antilipid drug	77±37.4	0

*Student's t-test; ^bYates continuity correction test; Statistical significance: *p<0.05; **p<0.01.

BMI: Body mass index; CRP: C-reactive protein; HbA1c: Glycated hemoglobin; HDL: High-density lipoprotein; LDL: Low-density lipoprotein.

Statistical Analyses

Number Cruncher Statistical System (NCSS, 2007; NCSS, LLC, Kaysville, UT, USA) and Power Analysis and Sample Size (PASS, 2008; NCSS, LLC, Kaysville, UT, USA) software were used for statistical analysis. The Student's t-test was used to analyze normally distributed variables among the clinical and biochemical characteristics of participants. Due to the number of subjects, the Kruskal-Wallis test was used to analyze more than 3 groups that were non-normally distributed. The Yates correction for continuity was used to compare qualitative data. Associations between continuous variables were analyzed with the Pearson correlation analysis. A p value <0.05 was considered statistically significant.

Results

A total of 240 participants were included in this study. The average duration of DM in the diabetic group was

7.51±7.43 years. For 78.2%, duration was less than 10 years, and for 21.8% it was greater than 10 years. The clinical and biochemical characteristics of the patients and controls are provided in Table 1. The 25(OH) D level was significantly lower in the T2DM patients compared with the healthy controls (11.16±3.99 ng/mL vs. 15.58±3.16 ng/mL; p<0.05). The T2DM patients had a significantly higher BMI, waist circumference, systolic pressure, fasting plasma glucose, HbA1c, parathyroid hormone, and CRP level than the controls (p<0.05 or p<0.01 for all). There were no significant differences between the T2DM patients and the control subjects in the other variables. Among the patients, 60.1% were using only oral antidiabetic drugs, 23.8% were using an oral antidiabetic drug plus insulin, and 6.3% were using insulin only. On the whole, 37.4% of the patients were taking lipid lowering drugs, and 44.2% were taking antihypertension drugs. The Pearson correlation analysis indicated that the serum 25(OH) D level showed a positive correlation with BMI (r= -0.129; p<0.048) and the HbA1c level (r=0.129;

Table 2. Relationship between vitamin D and other variables

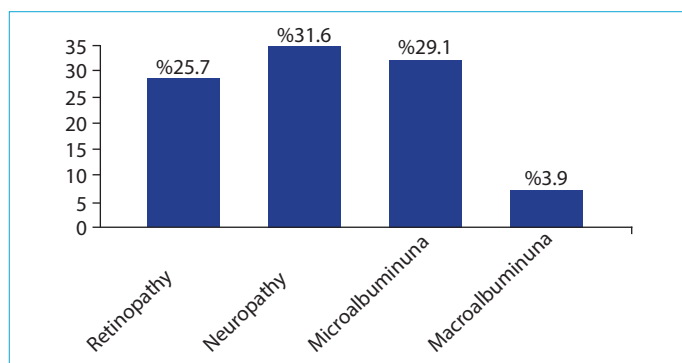
	25-OH vitamin D	
	R	P
BMI	-0.129	0.048*
HbA1C	0.129	0.045*
Parathyroid hormone	-0.170	0.015*

Pearson correlation * $p < 0.05$; BMI: body mass index; HbA1C: glycated hemoglobin.

Table 3. The effect of vitamin D on complications

		n	25-OH vitamin D (ng/mL)		^a p
			Median	SD	
Retinopathy	+	53	23.31	6.75	0.026
Neuropathy	+	65	23.15	7.42	0.039
Microalbuminuria	+	60	24.10	7.31	^b 0.286

^aStudent's t-test; ^bKruskal-Wallis test.

**Figure 1.** Distribution of complication prevalence.

$p < 0.045$), and a negative correlation with the parathyroid hormone level ($r = -0.170$; $p < 0.015$) (Table 2). As shown in Figure 1, among T2DM patients, 25.7% had DR ($n = 53$), 31.6% had neuropathy ($n = 65$), and 29.1% of them had microalbuminuria ($n = 60$).

To further examine the relationship between the serum 25(OH) D level and microvascular complication status, the Kruskal-Wallis test and the Student's t-test were also applied. There were statistically significant differences among the retinopathy, neuropathy, and 25(OH) D levels in T2DM ($p < 0.05$). However, there was no significant association between microalbuminuria and 25(OH) D level (Table 3).

Discussion

Microvascular complications of DM are associated with severe morbidity, mortality, and a huge economic burden. Although maintaining blood glucose and glycosylated hemoglobin concentrations as close to the normal range as possible would prevent or delay long-term complica-

tions,^[14] The Turkish Diabetes Epidemiology-II (TURDEP-II) population research revealed that in Turkey, the diabetes prevalence was 13.7% and that glycemic control was insufficient^[15] Factors contributing to the prevalence of diabetes in Turkey include undertreatment^[16]; a lack of patient compliance with treatment; and insufficient control of modifiable risk factors, such as body weight, physical inactivity, and diet.^[17] Research indicates that diabetes prevention focusing on change-of-lifestyle interventions is likely to be cost-saving.^[18]

In light of new information that vitamin D might improve insulin sensitivity and secretion^[19], we aimed to investigate the relationship between vitamin D and metabolic parameters and microvascular complications in T2DM patients. For this purpose, this study evaluated each of the 206 T2DM patients for microvascular complications and vitamin D level, in addition to routine metabolic parameters. In accordance with the existing literature, we found significantly high vitamin D levels in the T2DM patients. There are various potential mechanisms that link vitamin D with glucose metabolism.

Animal experiments have shown that vitamin D stimulation of beta-islet cells results in an increase of both cytosolic and intracellular calcium levels, as well as insulin secretion.^[20, 21] Insulin exocytosis from beta-cells is a calcium-dependent process. Vitamin D influences the extracellular and intracellular calcium balances, and deficiency supposedly plays a role in insulin secretion defects.^[22] Furthermore, recent studies suggest that vitamin D supplementation has beneficial effects on glucose homeostasis and glycemic control.^[23, 24] However, there are limited data on what the optimum vitamin D level might be, and still no consensus on the dose of vitamin D that should be administered to achieve a 25(OH) D concentration of 20 or 30 ng/mL. Different cut-off values for vitamin D have been suggested in literature.^[25, 26] The National Academy of Medicine (formerly called the Institute of Medicine, IOM), accepts a value between 20 and 30 ng/mL as insufficiency and less than 20 ng/mL as deficiency.^[27] In our study, the cut-off value of 20 ng/mL was used in correlation analysis based on these values. Our results indicated that the mean 25(OH) D concentration of the T2DM patients was 11.16 ng/mL, which is considered vitamin D deficiency. We also found that the mean was only 15.58 ng/mL in the control group, which suggests an unexpectedly high prevalence of vitamin D deficiency in the healthy population of a country with such a sunny climate.

The HbA1c level is considered one of the best clinical indicators of glycemic control and enabled an estimation of average blood glucose concentration during the preceding 2 to 3 months of the study.^[28] Zhou et al.^[29] recorded lower

HbA1c levels after vitamin D and calcium supplementation in T2DM patients. We found a positive correlation between 25(OH) D and HbA1c levels, suggesting that vitamin D may play a role in glycemic control of DM patients.

Vitamin D deficiency has recently been gaining acceptance as a risk factor for a decline in renal function in DM patients.^[30] Furthermore, a meta-analysis demonstrated that vitamin D can ameliorate proteinuria and protect kidneys from injury in DM patients, independently from blood pressure and glucose reduction.^[31] In light of these data, we looked for an association between vitamin D insufficiency and DN, but our results did not suggest this relationship with either microalbuminuria or macroalbuminuria. In our study, vitamin D level was not related to microvascular complications in significant numbers, similar to the findings of Suzuki et al.^[12], which may be explained by the small size of the sample group and by the participation in the study of patients who had serum a creatinine level of less than 2mg/dL.

Recent studies have revealed a close relationship between vitamin D and diabetic retinopathy. Payne et al.^[32] demonstrated significantly low vitamin D levels in T2DM patients with diabetic proliferative retinopathy. Similarly, Patrik et al.^[33] suggested a link between vitamin D prevalence and DR; however, vitamin D was not related to retinopathy severity. Although the exact mechanism between vitamin D deficiency and retinopathy remains unclear, it is hypothesized that vitamin D may act as a neuroprotective component for the optic nerve.^[34] Such a mechanism is accepted as a potent inhibitor for angiogenesis, and thus exerts an effect on the progression of DR.^[35] Seven of our patients were diagnosed with DR, and consistent with the studies mentioned above, the vitamin D level of these patients was significantly different from the other diabetic patients. In contrast to our findings, Bonakdaran et al.^[36] did not find any association between DR and its severity and vitamin D insufficiency, which may be explained by the high percentage of vitamin D insufficiency and the possibility of gene polymorphism.

Vitamin D has been suggested to be a neurotropic hormone; however, its role in diabetic neuropathic pain is still unclear.^[37] Vitamin D may interfere with nociceptor functions by causing diabetic nerve damage, which results in a decrease in the pain threshold in comparison with the nondiabetic population.^[38] Alamdari et al.^[39] demonstrated that a decreased level of circulating 25(OH)-D may contribute to an increased risk of large-fiber neuropathy in diabetic patients. Our data revealed that the vitamin D level was significantly lower in patients with diabetic polyneuropathy. However, interestingly, in a recent study, a non-linear contribution of serum vitamin D to symptomatic diabetic

neuropathy occurrence was reported, which suggests careful monitoring of vitamin D administration.^[40]

Similar to other studies, we found a significant negative relationship between the serum vitamin D level and the BMI of diabetic patients. In a large population study in the US, the serum 25(OH) D level was negatively associated with BMI.^[41] These findings may be explained by the deposition of vitamin D in fat tissue, thus decreasing bioavailability in obese patients.

There were some limitations to our study. Two prominent imitations are the small sample size, and the absence of information on the level of vitamin D in the diet. This study should be accepted as a pilot for further investigation of the impact of vitamin D on diabetic microvascular complications.

In conclusion, vitamin D insufficiency is more common in T2DM patients than the healthy population, and is associated with an increased risk of diabetic microvascular complications. Our study only showed such an association for nephropathy. In light of these data, T2DM patients should be screened for vitamin D deficiency. However, further large population studies on the effect of vitamin D supplementation on diabetic microvascular complications should be encouraged.

Disclosures

Ethics Committee Approval: The study protocol was approved by the Bakirkoy Sadi Konuk Research and Education Hospital Ethics Committee, Istanbul.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship contributions: Concept – B.B., A.H., G.S.; Design – B.B., A.H., G.S.; Supervision – A.H., D.S., B.B., A.M.; Materials – A.H., D.S., B.B., A.M., A.Y.; Data collection &/or processing – A.H., D.S., B.B., A.M., A.Y.; Analysis and/or interpretation – B.B., G.S., E.I., C.A.; Literature search – A.H., B.B.; Writing – A.H., B.B.

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