IMPACT OF VITAMIN D DEFICIENCY ON COGNITIVE FUNCTIONS IN TYPE 2 DIABETIC PATIENTS

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Abstract

Objective. We hypothesized that vitamin D-deficient diabetic patients might demonstrate cognitive deficits and therefore investigated this association in patients with diabetes mellitus (DM).

Methods. This study comprised 236 patients with type 2 DM (118 with vitamin D deficiency (less than 20 ng/mL) and 118 without vitamin D deficiency as the control group). All patients were outpatients in the endocrinology clinics of Baskent University, Turkey from 1 October 2013 to 31 January 2014 and, chosen in a randomized manner according to their application time. The serum vitamin D levels were measured and cognitive functions were evaluated using the Mini Mental Status Examination (MMSE).

Results. The mean age in the study and control group was 57.02±7.30 and 58.92±7.58 years, respectively (p=0.051). The male:female ratio in the study and control group was 1.00:1.46 and 1.00:2.11, respectively. The study group was further divided into three subgroups: patients with mild, moderate, and severe vitamin D deficiency. The study outcomes revealed no significant relationship between the vitamin D deficiency level and the total score or subscores of the MMSE (p<0.05).

Conclusions. These study results do not confirm the hypothesis that vitamin D deficiency unfavorably affects cognitive functions in patients with type 2 DM.

Key words: Vitamin D, Diabetes Mellitus, Cognitive Impairment, Cross-Sectional Study.

INTRODUCTION

During the 21st century, about 1 billion people were diagnosed with vitamin D deficiency worldwide, and that figure is on the rise (1). Vitamin D deficiency is thus a significant public health issue. It is diagnosed by measuring the serum 25(OH) cholecalciferol level. Many studies have shown that a low serum 25(OH) D level may be related to cognitive dysfunction and dementia (2-5). Moreover, some clinical studies have suggested that vitamin D replacement has a beneficial

impact on cognitive functions (6, 7). Animal studies and *in vitro* experiments have demonstrated the neuroprotective effects of vitamin D (8, 9). Former studies have determined an increased risk of cognitive impairment in older persons with longstanding low 25(OH)D levels. Evidence has also suggested that these individuals may develop dementia or become susceptible to cognitive dysfunction (10, 11). Mounting evidence from previous studies has consistently shown that vitamin D deficiency also plays a role in the pathophysiology of type 2 diabetes mellitus (DM) (12-19). The reverse is also true in that patients with type 2 DM have a higher prevalence of vitamin D hypovitaminosis due to multiple factors. Additionally, the 25(OH)D concentration is lower in patients with than without type 2 DM, a phenomenon that is similar to a higher prevalence of vitamin D hypovitaminosis in persons with type 2 DM (20-22). Diabetes, itself long-term complications are studied, is a chronic disorder leading to cognitive decline (23). It has become an important public health issue worldwide owing to its increasing prevalence. Thus, the potency and nature of the relationship among DM, cognitive dysfunction, and dementia should be covered in detail.

The impact of vitamin D on DM and cognitive functions and the interactions among these factors have recently been the subject of intense research (24-30). Some studies claim that patients with DM are at increased risk of developing cognitive impairment and that there is little evidence to suggest a relationship between cognitive impairment and treatment modalities or the duration of DM (25, 26). However, Chen *et al.* (27) demonstrated that the development of cognitive decline in patients with type 2 DM is associated with the level of 25(OH)D and that the level of vitamin D may predict major cognitive decline in these patients. In another systematic review of 13 studies from 1979 to 2008, Annweiler *et al.* (28) claimed that the relationship between the vitamin D level and cognitive performance

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remains uncertain and is in need of further study.

The most plausible reasons for such a relationship between cognitive functions and vitamin D levels involve the antioxidative effect of vitamin D and the presence of vitamin D receptors in the hippocampus. Accordingly, some researchers have claimed that vitamin D is needed for normal cognition and that deficiency of this vitamin predisposes to the development of diseases characterized by cognitive decline, such as Alzheimer's disease (31). Unfortunately, this hypothesis has not been supported by well-designed randomized controlled trials (RCTs). Furthermore, some RCTs have suggested no association between vitamin D levels and cognitive impairment (32).

Although this issue has been investigated in several studies, the effects of vitamin D deficiency on cognitive function in patients with type 2 DM remain unclear. In this study, we investigated whether hypovitaminosis D is associated with the clinical manifestation of decreased cognitive function in patients with type 2 DM.

PATIENTS AND METHODS

This cross-sectional study was conducted in patients aged 30 to 70 years with type 2 DM and without any other endocrinopathies who applied to the endocrinology outpatient clinics of Baskent University Ankara Hospital, Turkey from 1 October 2013 to 31 January 2014.

Ethical approval

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. This research project is approved by The Research and Ethics Committee of Baskent University Faculty of Medicine with the project number KA13/271.

Informed consent

Informed consent was obtained from all individual participants included in the study. The patients' blood vitamin D levels were measured only during this 4-month period to avoid the effects of sunlight during different seasons. During the study period, 907 patients aged 30 to 70 years with type 2 DM were admitted to our outpatient clinics. The power analysis for this study resulted in a study group of 118 patients and a control group of 118 patients to achieve a test power of 80%. All patients were randomly chosen

among applicants to the endocrinology outpatient clinics. In total, 236 patients with type 2 DM who had undergone measurement of their vitamin D level were enrolled in the study. The patients included in the study had a history of ≥ 1 year with type 2 DM, were aged 30 to 70 years, underwent regular follow-up in our clinics, and did not use vitamin D for at least 6 months prior to the blood testing.

The study participants were separated into two groups. Group 1 was the study group and comprised diabetic patients with vitamin D deficiency (25(OH)D < 30 ng/mL). Group 2 was the control group and comprised diabetic patients with normal vitamin D levels (25(OH)D > 30 ng/mL). Group 1 was further categorized into subgroups based on the severity of vitamin D deficiency: patients in the mild deficiency subgroup had a vitamin D level of 20 to 30 ng/mL, those in the moderate deficiency subgroup had a level of 10 to 20 ng/mL, and those in the severe deficiency subgroup had a level of < 10 ng/mL (33).

The levels of 25-hydroxyvitamin D3 (ng/mL) were analyzed by a high-performance liquid chromatography method with The Abbott Architect C δ 8200 system and the results were recorded. This method has a detection limit range from 0 ng/mL to 160 ng/mL. Insufficient levels of 25-hydroxyvitamin D3 was defined as less than 30 ng/mL. Serum creatinine level was assayed by the kinetic Jaffe method, rate-blanked and compensated; serum triglyceride, LDL-cholesterol, calcium levels were assayed by colorimetric method and serum B12 level was assayed by Chemiluminescent microparticle Intrinsic assay method using an Abbott® Architect C8000 and δ 2000 Analyzer according to the manufacturer's specifications (Abbott Park, IL, USA). HbA1c level was assayed fully automated direct enzymatic method from whole blood on the Abbott Architect chemistry system (Abbott Park, IL, USA). The calibration of the analyzer has been performed using Abbott calibrators (two levels, Ref. 4P52-02) constituted by whole blood samples with NGSP-assigned and IFCC-assigned HbA1c values.

Patients with dementia, Alzheimer's disease, or other neurological disorders were excluded from the study. Additionally, patients with a history of psychiatric disease, those who were under treatment with antidepressants or antipsychotic agents, and those who exhibited alcohol abuse were excluded. Patients with disorders affecting vitamin D metabolism such as chronic renal failure or any other endocrinopathies were not included in the study. Also, patients who

use drugs affecting bone and vitamin D metabolism such as multivitamin and/or calcium supplementation, bisphosphonates, corticosteroids, etc. were excluded from the study. All patients were randomly chosen among applicants with an appropriate diagnosis at the outpatient clinics.

Data regarding the patients' age, sex, education level, income level, dyslipidemia, duration of DM, and medications for DM (oral antidiabetics, insulin, or combination therapy) were collected. The study data were obtained by evaluating the available blood profile results and by filling out a questionnaire. We used the Mini Mental Status Examination (MMSE) to evaluate each patient's cognitive status. We used the MMSE to assess cognitive functions because its reliability and validity in the Turkish language have been proven and it is an easy-to-use test. The questionnaire was filled out by the investigators during a face-to-face interview with the patients (34,35).

The MMSE comprises 19 items in 5 categories to evaluate the mental status of a patient. This test provides information about a patient's orientation, registration, attention and calculation, recall, and language skills. The maximum score is 30 points; 27 to 30 points indicates a normal cognitive state (normal), 24 to 27 points indicates mild cognitive dysfunction (mild dementia), and <24 points suggests progressive

cognitive impairment (severe dementia). This test can distinguish patients with undetermined organic brain syndrome or depression from normal individuals. It is also known to be beneficial in measuring cognitive changes and impairment.

STATISTICS

All continuous data were tested for normality using the Kolmogorov–Smirnov test. Numerical variables with a normal distribution are expressed as mean $\pm$ standard deviation, numerical variables with a non-normal distribution as median (minimum–maximum), and categorical variables as number and percentage. Comparisons between variables were performed using Student's t test or the chi-square test. A stepwise multiple linear regression analysis was performed to identify significant predictors of MMSE scores. The study data were analyzed with SPSS for Windows 20.0 (IBM Corp., Armonk, NY). A p value of <0.05 was considered statistically significant.

RESULTS

The study population comprised 236 patients, 118 of whom were diagnosed with vitamin D deficiency (Group 1) and 118 with normal vitamin D levels (Group

Table 1. Distribution of demographic characteristics between the study and control groups

		Group 1	Group 2
Sex	Female	70 (59.3)	80 (67.8)
	Male	48 (40.7)	38 (32.2)
	Total	118 (100.0)	118 (100.0)
	$X^2=1.83$, $p=0.18^\dagger$		
Education	Primary education	16 (13.6)	14 (11.9)
	Secondary education	35 (29.6)	37 (31.3)
	University	67 (56.8)	67 (56.8)
	Total	118 (100.0)	118 (100.0)
Active hand	$X^2=2.31$, $p=0.51^\dagger$		
	Right	110 (93.2)	107 (90.7)
	Left	8 (6.8)	11 (9.3)
	Total	118 (100.0)	118 (100.0)
Duration of DM	$X^2=0.52$, $p=0.47^\dagger$		
	<10 years	81 (68.6)	88 (74.6)
	>10 years	37 (31.4)	30 (25.5)
	Total	118 (100.0)	118 (100.0)
Treatment of DM	$X^2=1.02$, $p=0.31^\dagger$		
	Oral antidiabetic	95 (80.5)	96 (81.3)
	Insulin	6 (5.1)	8 (6.8)
	Combination therapy	17 (14.4)	14 (11.9)
	Total	118 (100.0)	118 (100.0)
	$X^2=0.58$, $p=0.75^\dagger$		

Data are presented as n (%).

Group 1: Vitamin D-deficient group (study group)

Group 2: Group without vitamin D deficiency (control group)

† Chi-square test was used for evaluation of intergroup variables. No significant differences were found in the analyzed variables between Groups 1 and 2.

2). Group 1 was further divided into three subgroups: mild, moderate, and severe vitamin D deficiency. The mean age of the study population and control group was 57.02 ± 7.30 (33–69) and 58.92 ± 7.58 (34–68) years, respectively. Groups 1 and 2 were similar with respect to the mean age of the patients ($p=0.051$, t test). The demographic characteristics of the two study groups are shown in Table 1. The male:female ratio in the whole study group, Group 1, and Group 2 was 1.00:1.74, 1.00:1.46, and 1.00:2.11, respectively. Both groups were homogeneous regarding age, sex, educational status, active hand use, duration of DM, and DM treatment regimens. General laboratory parameters of the patients in both groups are shown in Table 2 and were not significantly different. The severities of vitamin D deficiency and patients distributed to each subgroup are shown in Table 3; the relationship of the duration of DM with the severity of vitamin D deficiency is also demonstrated. When analysing the groups according to the duration of DM, we found no

significant difference in either total vitamin D deficiency or the severity of deficiency between Groups 1 and 2. The mean HbA1c concentrations of the patients in Groups 1 and 2 were $6.89\% \pm 1.19\%$ (5.1%–10.6%) and $6.74\% \pm 1.18\%$ (5.0%–12.5%), respectively, and did not differ between the two groups. The MMSE scores and the mean HbA1c concentrations of the subgroups are shown in Table 3. The multiple linear regression analysis was adjusted for age, sex, and educational status. The results showed that the MMSE scores were significantly correlated with the educational status ($\beta=0.429$, $t=6.200$, $p=0.000$). Active hand use, the 25(OH)D level, the HbA1c concentration, and the vitamin B12 level were independently associated with the MMSE scores.

The mean MMSE scores for each cognitive function category are compared between the two groups in Table 4. No correlation between vitamin D deficiency and cognitive functions was found with respect to the duration of diabetes, sex, active hand use,

Table 2. Comparison of age and laboratory parameters between two groups

	Group 1	Group 2	p value*
Age (years)	57.02 ± 7.30	58.92 ± 7.58	0.051
25(OH)D (ng/mL)	17.20 ± 6.53	39.90 ± 11.86	0.000
HbA1c (%)	6.89 ± 1.20	6.74 ± 1.18	0.354
LDL-C (U/L)	124.19 ± 31.52	123.91 ± 32.17	0.948
TG-C (mg/dL)	164.49 ± 130.00	159.63 ± 68.63	0.731
Ca (mg/dL)	9.50 ± 0.50	9.44 ± 0.50	0.470
Cr (mg/dL)	0.85 ± 0.19	0.82 ± 0.20	0.180
Vitamin B12 (pg/mL)	452.82 ± 326.95	452.12 ± 222.25	0.987

Data are presented as mean $\pm$ standard deviation.

LDL, low-density lipoprotein; TG-C, triglycerides-cholesterol; Ca, calcium; Cr, creatinine

Group 1: Vitamin D-deficient group (study group)

Group 2: Group without vitamin D deficiency (control group)

*Student's t test was used for evaluation of intergroup variables.

Table 3. Statistical evaluation according to vitamin D level, duration of DM, HbA1c concentration, and MMSE score

		Group 1			Group 2	p value
		Mild	Moderate	Severe		
Vitamin D level, (ng/mL)		50 (21.2)	54 (22.9)	14 (5.9)	118 (50.0)	
		23.3 ± 2.6	14.6 ± 3.1	5.4 ± 1.7	17.2 ± 6.5	39.9 ± 11.9
DM duration						
	<10 years	36 (72.0)	35 (64.8)	10 (71.4)	81 (68.6)	88 (74.6)
	>10 years	14 (28.0)	19 (35.2)	4 (28.6)	37 (31.4)	30 (25.4)
HbA1c, %						
		6.57 ± 1.02	7.10 ± 1.33	7.12 ± 1.06	6.89 ± 1.19	6.74 ± 1.18
MMSE score						
		28.50 ± 1.81	27.94 ± 2.61	27.29 ± 2.53	28.10 ± 2.31	28.33 ± 1.84

Data are presented as n (%) or mean $\pm$ standard deviation.

MMSE, Mini Mental State Examination

Group 1: Vitamin D-deficient group (study group)

Group 2: Group without vitamin D deficiency (control group)

*There was no significant difference among the subgroups of the vitamin D deficiency.

**There was no significant difference between Groups 1 and 2.

aStudent's t test, bone-way analysis of variance, cthe chi-square test, and dthe Mann–Whitney U test were used for evaluation of intergroup variables.

Table 4. MMSE cognitive function scores in patients with and without vitamin D deficiency

Categories of MMSE	Group 1*	Group 2**	p value
Orientation	9.89±0.61	9.97±0.16	0.301
Registration memory	3.00±0.00	3.00±0.00	1.000
Attention - making an account	4.39±1.40	4.40±1.37	0.916
Remember	2.19±0.75	2.17±0.85	0.863
Language	8.65±0.59	8.79±0.43	0.080
Total score	28.10±2.31	28.33±1.84	0.616

Scores are presented as mean ± standard deviation. MMSE, Mini Mental State Examination.

*Group 1: Vitamin D-deficient group (study group)

**Group 2: Group without vitamin D deficiency (control group)

or educational status. The total test score and subscore analyses based on the severity of vitamin D deficiency revealed no significant correlation between vitamin D deficiency and cognitive functions.

DISCUSSION

Vitamin D has recently become a popular scientific research subject (24). Many epidemiological studies have shown that the risk of DM and cognitive dysfunction increases with vitamin D deficiency. The rate of vitamin D deficiency is also reportedly higher among patients with type 2 DM (25-29). Allen *et al.* (25) reviewed 10 studies exploring the relationship between DM and cognitive functions (9 population-based studies and 1 case control study) and found evidence of a possible link between DM and both cognitive decline and dementia types. Later, a systematic review by Biessels *et al.* (26) provided strong evidence of an increased risk of dementia in the general population of patients with DM. A similar risk increment was also shown for patients with Alzheimer's disease (26). In the prospective Doetinchem Cohort Study conducted in 2013 in Holland (23), 2613 participants underwent cognitive function assessment twice within 5 years. The cognitive decline was 2.6 times more common in patients with than without DM. Alencar *et al.* (35) reported that the MMSE could be useful for the assessment of cognitive functions in patients with type 2 DM.

Clinical studies have confirmed that patients with DM have a higher prevalence of hypovitaminosis D due to many factors (20-22). In patients with type 2 DM, the HbA1c concentration is higher when hypovitaminosis D is present. Additionally, genetic changes in vitamin D receptors are associated with an increased risk of DM. A recent cross-sectional study conducted in Tehran, Iran detected a higher prevalence of vitamin D deficiency in patients with than without type 2 DM (37).

Vitamin D receptors have been detected in many cells, and they are considered to play a role in the

cell cycle and regulation of organ functions. Vitamin D may provide a neuroprotective effect and help prevent neurodegeneration in the central nervous system. In light of these data, some studies have suggested that low serum 25(OH)D levels may be related to cognitive impairment and dementia (28-30). Van der Schaft *et al.* (30) reviewed 2182 articles in the Embase and PubMed databases in June 2012 to investigate the presence of a relationship between serum vitamin D levels and cognitive functions. They excluded 2151 articles that did not meet the inclusion criteria and analysed 25 cross-sectional and 6 prospective studies. They found that in 18 (72%) of the 25 cross-sectional studies, a low vitamin D level was associated with worse cognitive function test results and an increased risk for dementia, while 7 (28%) studies concluded that there was no such relationship. In four of the prospective studies, the risk of cognitive decline increased in patients with vitamin D deficiency over 4 to 7 years of follow-up (30). These findings have not been supported by well-designed RCTs until now (32).

A retrospective study from the United States investigated vitamin D and B12 levels and applied the MMSE in 80 patients in a memory clinic. A positive correlation was found between the serum 25(OH)D level and MMSE scores ($p=0.0006$), whereas no significant relationship was shown between the vitamin B12 level and MMSE scores ($p=0.875$) (5). We demonstrated no correlation between vitamin D deficiency and MMSE scores in both the total test score and subscore analyses performed based on the severity of vitamin D deficiency. Some studies similarly suggested that there is a weak or virtually no relationship between low serum 25(OH)D levels and cognitive decline (2-5). A randomized clinical study of healthy young adults in Queensland, Australia compared subjects receiving vitamin D replacement and subjects receiving placebo with respect to cognitive and emotional functions. No impact of vitamin D replacement was found (38).

In this study, the number of control group patients was higher than expected, because of the fact

References

that our country is located in the Mediterranean area, which is characterized by a sunny climate during at least eight months a year. Accordingly, both study and control groups in the present study were exposed to similar amounts of daylight with almost the same UV index. Both groups were homogenized with regard to confounding factors such as age, sex, duration of DM, treatment for DM, educational status, and active hand use, which distinguishes the present study from similar studies.

LIMITATIONS

Unfortunately this study has several limitations. Firstly, vitamin D levels show a seasonal pattern, and the lowest UV index in the city of Ankara occurs from October to December. We may have identified some patients with current vitamin D deficiency who were otherwise non-deficient during the rest of the year. Furthermore, the lack of information on the duration of vitamin D deficiency at the time of application to the outpatient clinic and how treatment may have improved these outcomes could not be clearly identified. Accordingly, this study included only recently measured vitamin D levels. Therefore, we do not know how long each patient was exposed to vitamin D deficiency. Another limitation was that factors such as the duration of DM, education level, and active hand use are dependent upon the patients' self-reports, and therefore may lead to recall bias. One of the most important limitations of the study was its limited sample size. In particular, the size of the control group was somewhat inadequate to identify possible confounders.

In conclusion, this study was designed based on the hypothesis that vitamin D deficiency unfavourably affects cognitive functions. It explored to what extent cognitive functions are affected by vitamin D deficiency in patients with DM and how DM affects cognitive functions. The study explored whether there is an association between vitamin D levels and cognitive function in patients with type 2 DM, but did not demonstrate any significant relationship. This result was not altered by whether the duration of DM was >10 or <10 years, the patients' sex, or the patients' educational status. The total test score and subscore analyses based on the severity of vitamin D deficiency revealed no significant correlation between vitamin D deficiency and MMSE scores.

Conflict of interest

The authors declare that they have no conflict of interest.

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