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Vitamin D status and its association with gradual decline in cognitive function

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Background/aim: To compare plasma vitamin D concentrations among patients with normal cognitive function (control group), mild cognitive impairment (MCI), and Alzheimer disease (AD).

Materials and methods: In total, 158 patients with AD, 228 patients with MCI, and 603 control subjects were included. Plasma levels of 25-hydroxyvitamin D were measured after comprehensive geriatric assessment and compared among groups. SPSS 15.0 was used for statistical analysis.

Results: Mean levels of 25-hydroxyvitamin D were significantly different among the 3 groups of AD patients, MCI patients, and controls ($P < 0.001$). Post hoc analysis revealed that the levels were significantly lower in the MCI group than the control group ($P = 0.002$) and significantly lower in the AD group than the control group ($P = 0.003$). Multivariate analysis showed that age (OR: 1.070, 95% CI: 1.025–1.116, $P = 0.002$), instrumental activities of daily living score (OR: 0.920, 95% CI: 0.850–0.995, $P = 0.037$), 25-hydroxyvitamin D level (OR: 0.959, 95% CI: 0.932–0.987, $P = 0.004$), and diabetes mellitus (OR: 2.476, 95% CI: 1.153–5.319, $P = 0.020$) were factors independently associated with AD.

Conclusion: This study demonstrated that there is a correlation between plasma 25-hydroxyvitamin D levels and cognitive functions.

Key words: Vitamin D status, Alzheimer disease, cognitive dysfunction, minimal cognitive impairment

1. Introduction

Alzheimer disease (AD) is the most common form of dementia in geriatric patients and progresses with progressive memory loss and cognitive dysfunction (1,2). Vitamin D is one of the essential hormones for homeostasis, muscle strength, and innate immunity. It maintains many physiological functions (3). Vitamin D deficiency is associated with increased risk of several types of cancer, autoimmune diseases, and cardiovascular disorders (4). In the last decade, experimental studies demonstrated that vitamin D is the 'forgotten neurosteroid' hormone required for normal brain regulation and development (5). Vitamin D plays an important role in neurodegenerative disorders with pathogenesis related to neurotrophin, inducible nitric oxide synthesis, glutathione and monoamine synthesis, and apoptosis (6).

Low serum 25-hydroxyvitamin D levels are frequently determined among geriatric patients with a prevalence reaching 90% (7). Hypovitaminosis D is also highly associated with cognitive disorders, especially with advanced-stage dementia (8,9). Mild cognitive impairment (MCI) is the early stage of cognitive dysfunction, causing memory complaints without a disturbance in daily life activities, and is encountered with an increasing prevalence of AD (10). Although there are some studies examining the relationship between cognitive decline and vitamin D levels, to the best of our knowledge, it is not well documented and there is no consensus in the literature about the relationship between serum 25-hydroxyvitamin D level and patients' cognitive status. The aim of this current study is to measure vitamin D concentrations and evaluate its association with cognitive functions among 3 groups of patients with normal cognitive function, MCI, and AD.

2. Materials and methods

2.1. The participants and study design

A total of 989 subjects aged 65 years and older who were admitted to our outpatient clinic of geriatric medicine

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were included in this cross-sectional study. To evaluate the participants, comprehensive geriatric assessment, including the evaluation of medical history, physical examination, and assessment scales such as the Mini-Mental State Examination (MMSE) (11), Activities of Daily Living (ADL) (12), Mini Nutritional Assessment Short Form (MNA-SF) (13), and Instrumental Activities of Daily Living (IADL) (14) scales, was performed. DSM-IV (15) and NINCDS-ADRDA (16) criteria were used for the diagnosis of AD. Neuroimaging by using magnetic resonance was performed for patients before AD diagnosis in order to exclude reversible causes of dementia. The patients with AD had a score of 1 or higher according to Clinical Dementia Rating (CDR) Scale (17). Peterson's criteria were used for MCI diagnosis (18–20). The participants of the control group with normal cognitive function had been selected from patients meeting the following criteria: age- and sex-matched to the AD and MCI groups, no memory complaints, normal test scores of MMSE and clock drawing tests (21), not meeting the criteria for MCI or AD, and score of 0 on the CDR Scale.

The medical histories of the patients were evaluated. Presence of hypertension, diabetes mellitus, coronary artery diseases (history of angina pectoris, previous heart attack, myocardial infarction, and documented coronary artery disease by coronary angiography), other systemic chronic diseases, and history of operations were noted. Body mass index (BMI) was calculated by the formula of body weight/height^2. Medications received by the patients were noted, including medications containing calcium or vitamin D. All the participants underwent standardized clinical examination.

Patients with diagnosis of renal failure, liver failure, and malignant diseases were excluded from the study.

This study was approved by the Local Ethics Committee of Hacettepe University Faculty of Medicine and was conducted in accordance with the Declaration of Helsinki.

The participants were divided into 3 groups: AD patients, MCI patients, and patients with normal cognitive function (control group).

2.2. Laboratory examination

Routine laboratory tests were performed in order to evaluate the patients and exclude reversible causes of dementia. Blood samples were collected after an 8-h fast. Routine hemogram laboratory tests including hemoglobin, white blood cell count, platelet levels, and biochemical analyses were performed. Biochemical tests consisted of vitamin B12, folic acid, total protein and albumin, fasting plasma glucose (FPG), creatinine, alanine aminotransferase (ALT), aspartate aminotransferase, thyroid stimulating hormone, alkaline phosphatase, gamma glutamyl transferase, total cholesterol (TC), high density lipoprotein, triglyceride, low density lipoprotein, and C-reactive protein (CRP) levels. Plasma 25-hydroxyvitamin D levels were assessed by high-performance liquid chromatography. The plasma vitamin D levels were measured using the LC-20AT solvent delivery unit (Shimadzu Corporation, Kyoto, Japan), and the ImmuChrom ELISA kit (ImmuChrom GmbH, Heppenheim, Germany) was used for analysis of plasma vitamin D levels. The intraassay coefficients of variation (CVs) were 2.6% (control value: 22.6 ng/mL) and 1.5% (control value: 41.9 ng/mL), the interassay CVs were 4.0% (control value: 21.6 ng/mL) and 3.6% (control value: 42.2 ng/mL), and the kit's detection limit and upper limit of linearity were 2.3 ng/mL and 500 ng/mL, respectively.

2.3. Statistical analysis

The distribution of continuous variables was assessed by visual and analytic methods such as histogram, Kolmogorov–Smirnov or Shapiro–Wilks tests, and probability plots. Normally distributed continuous variables are shown as mean ± standard deviation (SD), while skew-distributed continuous variables are shown as median (minimum–maximum). Categorical parameters are shown as number of patients and frequencies. Pearson's chi-square test was used for comparing categorical variables. Comparisons of normally distributed continuous variables among the 3 groups were evaluated by one-way ANOVA test. To determine the homogeneity of variances, the Levene test was used. The Tamhane test and post hoc Tukey tests were applied in accordance with homogeneity of variances. The Kruskal–Wallis test was used for comparing ADL and CRP levels. Pearson or Spearman tests were used for the correlation analysis of numerical variables. To define the independent related factors for AD, logistic regression analysis was performed. Values of $P < 0.05$ were considered statistically significant. For the statistical analyses, SPSS 15.0 was used.

3. Results

A total of 158 patients with AD, 228 patients with MCI, and 603 patients with normal cognitive function were included in this study. Mean age ± SD of subjects was 71.4 ± 6.3 years in patients with normal cognitive function, 71.7 ± 5.7 years in MCI patients, and 75.2 ± 6.7 years in AD patients ($P < 0.001$), while 229 (38%) of the control group, 61 (26.8%) of the MCI group, and 63 (39.9%) of the AD group were male ($P = 0.005$). Demographic properties, geriatric assessment test scores, laboratory parameters, and comorbidities of the study population are presented in Table 1.

The mean ± SD of 25-hydroxyvitamin D levels was significantly different among the 3 groups ($P < 0.001$) (Figure). After post hoc analysis, significant differences were found between MCI and control groups (20.68 vs. 23.74 ng/mL, $P = 0.002$) and AD and control groups (20.29 vs. 23.74 ng/mL, $P = 0.003$). There was no significant

Table 1. Demographic properties, geriatric assessment test scores, laboratory parameters, and comorbidities of the study population.

Parameters*	Control (n = 603)	MCI (n = 228)	AD (n = 158)	P
Age, years	**71.4 ± 6.3**	**71.7 ± 5.7**	**75.2 ± 6.7**	**<0.001**
Sex (male)	**229 (38.0%)**	**61 (26.8%)**	**63 (39.9%)**	**0.005**
BMI, kg/m^2	27.30 ± 4.06	26.81 ± 4.15	26.85 ± 3.45	0.336
MMSE score	**26.71 ± 3.65**	**25.97 ± 3.69**	**21.51 ± 7.38**	**<0.001**
ADL score	0.00 (0.00–24.00)	1.00 (0.00–8.00)	0.00 (0.00–27.00)	0.644
IADL score	**14.64 ± 3.00**	**14.46 ± 3.24**	**13.50 ± 3.97**	**0.016**
MNA-SF score	**12.29 ± 2.16**	**12.47 ± 1.81**	**11.41 ± 3.01**	**0.002**
Vitamin D, ng/mL	**23.74 ± 12.35**	**20.68 ± 11.07**	**20.29 ± 9.34**	**<0.001**
HGB, g/dL	13.82 ± 1.34	13.72 ± 1.22	13.73 ± 1.29	0.552
WBC, /μL	6741.10 ± 2049.06	6685.42 ± 1916.61	6782.21 ± 1868.60	0.890
PLT, /μL	252,919.40 ± 70,605.90	256,204.40 ± 70,579.88	249,309.70 ± 85,354.91	0.662
CRP, mg/dL	0.37 (0.10–20.31)	0.35 (0.10–20.10)	0.34 (0.10–12.00)	0.649
Vitamin B12, pg/mL	323.58 ± 183.89	338.93 ± 113.20	347.36 ± 139.03	0.342
Folate, ng/mL	11.84 ± 5.24	11.91 ± 5.58	10.79 ± 5.35	0.079
BUN, mg/dL	18.64 ± 5.64	18.25 ± 6.06	19.66 ± 6.15	0.062
Creatinine, mg/dL	**0.92 ± 0.27**	**0.87 ± 0.23**	**0.96 ± 0.30**	**0.004**
Total protein, g/dL	7.53 ± 0.47	7.52 ± 0.47	7.45 ± 0.56	0.219
Albumin, g/dL	4.23 ± 0.33	4.23 ± 0.34	4.18 ± 0.36	0.188
FPG, mg/dL	**109.19 ± 42.27**	**105.40 ± 38.15**	**97.57 ± 24.61**	**0.005**
TC, mg/dL	**208.17 ± 45.77**	**215.88 ± 46.20**	**203.23 ± 45.37**	**0.021**
TG, mg/dL	142.21 ± 72.22	142.73 ± 81.04	134.57 ± 66.32	0.478
HDL, mg/dL	55.53 ± 14.82	57.90 ± 14.14	56.78 ± 14.74	0.108
LDL, mg/dL	123.26 ± 38.65	125.61 ± 40.96	119.61 ± 36.30	0.333
TSH, μIU/mL	1.54 ± 1.26	1.54 ± 1.15	1.63 ± 1.24	0.702
ALT, U/L	**19.66 ± 8.89**	**20.19 ± 9.15**	**17.89 ± 7.76**	**0.034**
AST, U/L	21.92 ± 9.05	22.48 ± 8.56	22.03 ± 13.38	0.765
ALP, U/L	146.93 ± 82.43	147.93 ± 84.76	143.92 ± 85.79	0.893
GGT, U/L	25.78 ± 19.67	25.08 ± 21.18	22.30 ± 18.46	0.157
Season (summer)	361 (60.0%)	141 (62.1%)	79 (50.3%)	0.050
Vitamin D therapy	251 (41.60%)	85 (37.30%)	44 (31.90%)	0.085
HT	434 (72.20%)	170 (74.90%)	104 (67.10%)	0.246
DM	**151 (25.10%)**	**58 (25.60%)**	**12 (7.60%)**	**<0.001**
CAD	165 (25.10%)	52 (22.90%)	30 (19.20%)	0.072

MCI: Mild cognitive impairment, AD: Alzheimer disease, BMI: Body mass index, MMSE: Mini-Mental State Examination, ADL: Activities of Daily Living, IADL: Instrumental Activities of Daily Living, MNA-SF: Mini-Nutritional Assessment Test-Short Form, HGB: Hemoglobin, WBC: White blood cell, PLT: Platelet, CRP: C-reactive protein, BUN: Blood urine nitrogen, FPG: Fasting plasma glucose, TC: Total cholesterol, TG: Triglyceride, HDL: High density lipoprotein, LDL: Low density lipoprotein, TSH: Thyroid-stimulating hormone, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase, GGT: Gamma glutamyl transferase, HT: Hypertension, DM: Diabetes mellitus, CAD: Coronary artery disease. *: Parameters with statistically significant differences.

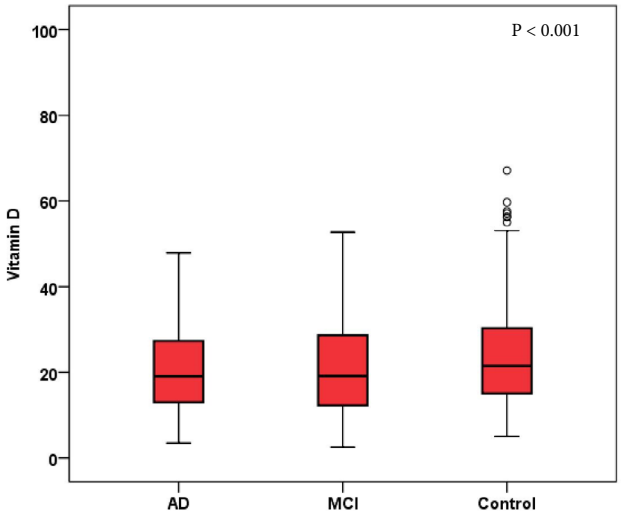

Figure. Plasma vitamin D levels (ng/mL) of the patients with AD, MCI, and normal cognitive functions (20.29 ± 9.34 ng/mL, 20.68 ± 11.07 ng/mL, and 23.74 ± 12.35 ng/mL, respectively). After post hoc analysis, significant differences were found between MCI and control groups (20.68 vs. 23.74 ng/mL, P = 0.002) and AD and control groups (20.29 vs. 23.74 ng/mL, P = 0.003).

difference between patients with MCI and AD (P = 0.944). The mean ± SD values of IADL score, MNA-SF scores, and FPG, TC, and ALT levels were significantly lower while creatinine was significantly higher in patients with AD than in the control group. Frequency of diabetes mellitus in AD patients was significantly lower than in the MCI and control groups. The mean ± SD of vitamin D levels of male patients was significantly higher than in female patients (24.22 ± 11.42 vs. 21.46 ± 11.74 ng/mL, P < 0.001). The number of male patients receiving vitamin D replacement was lower than female patients [93 (26.9%) vs. 289 (45.9%), P < 0.001].

Age, sex, MMSE score, IADL score, MNA-SF score, diabetes mellitus rate, and 25-hydroxyvitamin D, creatinine, FPG, TC, and ALT levels were significantly different among the 3 groups in univariate analysis. All of these parameters and the data on receiving vitamin D supplements were put into equations for multivariate analysis in order to find out the independent correlates of AD. Multivariate regression analysis revealed that age (OR: 1.070, 95% CI: 1.025–1.116, P = 0.002), IADL score (OR: 0.920, 95% CI: 0.850–0.995, P = 0.037), 25-hydroxyvitamin D level (OR: 0.959, 95% CI: 0.932–0.987, P = 0.004), and diabetes mellitus (OR: 2.476, 95% CI: 1.153–5.319, P = 0.020) were independent correlates of AD. The results of the multiple logistic regression analysis of the possible correlates for AD are summarized in Table 2.

Correlation analysis between plasma vitamin D levels and other biochemical parameters revealed that there were no correlations between vitamin D levels and BMI (P = 0.350, r = –0.036), blood urea nitrogen (P = 0.527, r = –0.020), protein (P = 0.950, r = 0.002), albumin (P = 0.076, r = 0.057), triglyceride (P = 0.956, r = 0.002), low density lipoprotein (P = 0.241, r = 0.038), very low density lipoprotein (P = 0.396, r = 0.027), total cholesterol (P = 0.156, r = 0.045), thyroid-stimulating hormone (P = 0.975, r = –0.001), CRP (P = 0.445 r = –0.026), and homocysteine levels (P = 0.700, r = –0.013), but there were very weak positive correlations between vitamin D levels and high density lipoprotein (P = 0.043, r = 0.065), vitamin B12 (P = 0.006, r = 0.090), folate (P = 0.038, r = 0.067), uric acid (P < 0.001, r = 0.125), and creatinine (P = 0.002, r = 0.101) levels.

4. Discussion

In this cross-sectional study, we found that 25-hydroxyvitamin D levels of patients with MCI and AD were significantly lower than those of patients with normal cognitive function and 25-hydroxyvitamin D was independently related with AD. To the best of our knowledge, this is the first study comparing these 3 groups in the same setting.

During the last decade, vitamin D and its constructive effects on the human body has been one of the big subjects of debate and investigation. As it is a common deficiency in the geriatric population, it is important to seek its potential role in cognitive dysfunction. First, a systemic

Table 2. The results of the multiple logistic regression analysis of the possible correlates for AD.

Parameters	β	95% CI		P
		Lower	Upper	
Age	1.070	1.025	1.116	0.002
IADL	0.920	0.850	0.995	0.037
Vitamin D	0.959	0.932	0.987	0.004
DM	2.476	1.153	5.319	0.020

IADL: Instrumental Activities of Daily Living, DM: Diabetes Mellitus.

review in 2009 revealed the association between cognitive problems and vitamin D deficiency (22). In spite of gradual incremental data regarding the association between vitamin D deficiency and cognitive problems, to the best of our knowledge it is still unclear whether serum vitamin D level is associated with cognitive status and whether it is significantly different in patients with normal cognitive function, MCI, and AD. On the other hand, evidence about only MCI in the literature is very weak. In one study, Wilkins et al. (23) chose only the Short Blessed Test (SBT) in order to compare patients, with only 60 MCI and normal cognitive function patients, and they found a relationship between vitamin D status and SBT score. A recent study about patients with MCI and normal cognitive function was done by Annweiler et al. (10), where 125 patients were included in the Gait and Alzheimer Interaction Tracking (GAIT) study and they were divided into 2 groups according to their cognitive status: 95 of 125 were nondemented and the remaining 43 patients had MCI. They reported that 25-hydroxyvitamin D levels were significantly lower ($P = 0.006$) in MCI patients and logistic regression analysis showed that higher vitamin D levels decreased the risk of MCI. Our study also demonstrated that vitamin D levels were significantly lower in 228 patients with MCI than in 603 patients with normal cognitive function ($P = 0.001$). One of the strengths of our study is the high number of patients analyzed.

Some genetic and nongenetic factors play roles in the main pathogenesis of AD. AD is the end point of degenerative and vascular processes. Progressive neuronal damage causes gradual cognitive decline. This multifactorial pathogenesis leads researchers to study multitarget solutions. At this point, vitamin D is a recently recognized popular multitarget neuroprotective agent. Vitamin D has effects via its receptor (VDR) on neurons in the way of crossing the blood brain barrier (24). In AD, amyloid β-42 peptide aggregates in the extracellular space as senile plaques. This process results in neuron death by the neurotoxic effects of amyloid beta through oxidative stress, inflammation, and excitotoxicity. Meanwhile, amyloid plaques and oxidative stress increase the phosphorylation of tau proteins controlled by the MAP kinase enzyme. Thus, neurofibrillary tangles aggregate and degeneration of neurons is reinforced (1). As a result, both amyloid plaques and neurofibrillary tangles lead to excessive calcium entry into neurons by glutamatergic neuronal stimulation, causing necrosis and apoptosis (25). Roles of vitamin D in the central nervous system include its neurotrophic, neuroprotective, and neuromodulating effects on those pathophysiologic mechanisms of AD (26). Furthermore, an animal study indicated that vitamin D usage increases the activity of choline acetyltransferase in different parts of the rat brains. This study supports the idea that hypothesis vitamin D may play a role on acetylcholine pathway (27). Genetic factors are more virgin fields of AD. Experimental studies shows that VDR genotypes and gene polymorphism may have an essential role in the neuroprotective effect of vitamin D (28).

The role of low levels of vitamin D as a vascular risk factor may be another hypothesis underlying this relationship. Recently, several studies demonstrated that 25-hydroxyvitamin D may be a novel marker for cardiovascular disease. Lower levels of 25-hydroxyvitamin D were found to be associated with all causes of mortality and higher myocardial infarction risk, whereas an increase in its level protects against cardiovascular disease (29–31). In the last decade, vascular factors have been shown to play roles in AD. Various vascular changes, atherosclerosis, and endothelial dysfunction were found to be linked to AD (32,33). This vascular hypothesis may be the link between AD and vitamin D. Another possible explanation for the significant association between vitamin D status and cognitive dysfunction was suggested in previous studies. It was suggested that AD and similar degenerative diseases may cause deprivation of feeding and sun exposure (4,8), thus leading to vitamin D deficiency. However, our results showing no significant difference in vitamin D levels between MCI and AD patients effectively demonstrates that vitamin D level may be low in patients without any functional decline, as in MCI. This makes the hypotheses of vitamin D acting as a neuroprotective agent and vitamin D deficiency being a vascular risk factor more possible mechanisms to explain this significant association.

In this study, we did not find a significant difference between MCI and AD groups. It was previously shown that continuous vitamin D deficiency is strongly associated with the progression of cognitive decline, as from MCI to AD (1). Diagnosis of MCI is still not clear enough. Different diagnostic criteria are used in different studies. The most feasible and most widely used criteria are the Peterson criteria, which were used in our study for MCI diagnosis. On the other hand, many of the patients with AD in the present study were in the early stage; the percentage of moderate and severe AD patients were very low. This pathophysiological process might be the reason for not finding a significant difference in vitamin D levels between MCI and AD groups (34). Further studies including more patients with moderate and severe dementia with longitudinal follow-up are needed to further explain this point.

Most of the patients in the control, MCI, and AD groups were female (n = 374 of 603, n = 167 of 228, and n = 95 of 158, respectively). In spite of some studies (35–37) showing that sex difference may have some effects on results, others demonstrated no effect of sex on the association of vitamin D status and cognitive functions (38). In our study, although the number of patients receiving vitamin

D therapy was lower in male patients, vitamin D level of male patients was higher than that of female patients. This difference may be due to the clothing habits of our female patients. As the majority of them are covered, this may interfere with exposure to sunlight. The recommended vitamin D level in patients in order to prevent adverse events is 30 ng/mL (39). In our study, the mean ± SD of vitamin D level in the AD group was 20.29 ± 9.34 ng/mL. Other independent correlates of AD were determined to be age, diabetes mellitus, and IADL score. Advanced age is a well-known contributing factor for AD (38,40). Diabetes mellitus as a cardiovascular risk factor significantly and independently increases risk for AD (41). IADL score is one of the important cornerstones of dementia and its progression also affects survival rates (42,43). The present study also emphasized that independency in IADL has a negative association with AD.

Our study has some potential limitations that should be indicated. Initially, it was done in one center and probably does not represent the general geriatric population. The cross-sectional design of our study was the biggest handicap, such that we may not determine causality. Furthermore, some factors that may interfere with vitamin D status, such as serum parathyroid hormone levels, the proportion of dietary intake of vitamin D, and genetic factors of VDR genotypes and polymorphism, were not studied.

In conclusion, our study demonstrated that there is a strong correlation between 25-hydroxyvitamin D levels and cognitive functions. Further prospective studies are required to explain this association. In addition, randomized clinical trials are essential to understand whether vitamin D treatment has an influence on prevention and amelioration of AD or not.

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