Vitamin D in mild cognitive impairment and Alzheimer's disease. A study in older Greek adults

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Abstract

Background: In recent years, accumulating evidence has linked vitamin D deficiency to cognitive dysfunction and dementia. This study aimed at determining the relevance of serum 25-hydroxyvitamin D concentrations in mild cognitive impairment (MCI) and Alzheimer's disease (AD) in older Greek adults. It also examined whether the vitamin D level could be considered a predisposing factor for conversion from MCI to AD.

Methods: The study enrolled 350 subjects aged 65 years and over, allocated into three groups consisting of 103 healthy subjects (HS), 109 individuals with MCI, and 138 patients with AD, respectively. Serum 25-hydroxyvitamin D [25(OH)D] concentrations, measured in ng/ml, were determined by electrochemiluminescence, and we used the Mini-Mental State Examination (MMSE) and the Cambridge Cognition Examination (CAMGOG) to evaluate the subjects' cognitive status. One follow-up examination was performed for the MCI patients 30 months ± three months after the initial evaluation.

Results: Compared to HS, serum 25(OH)D levels were significantly decreased in individuals with MCI (p =0.012) and patients with AD (p <0.001). Moreover, serum 25(OH)D concentrations were significantly decreased in patients with AD compared to individuals with MCI (p =0.003) and also significantly lower in individuals with MCI who progressed to AD compared to those who remained MCI (p =0.028). After adjusting for confounders, multivariate analysis revealed that an increase of vitamin D concentration by one ng/mL reduces the risk of MCI by 4 % (OR =0.96, 95 % CI =0.92-0.99, p =0.006), the risk of AD by 8 % (OR =0.92, 95 % CI =0.89-0.95, p <0.001), and in an individual with MCI reduces the risk of conversion to AD by 10 % (OR =0.90, 95 % CI =0.83-0.96, p =0.003).

Conclusions: The present study reveals that serum vitamin D levels are significantly decreased in subjects with MCI and patients with AD compared to HS. Additionally, individuals with MCI who progressed to AD presented significantly lower vitamin D levels than those who remained MCI. These results suggest that preserving adequate vitamin D status in older adults could delay or prevent cognitive decline. HIPPOKRATIA 2020, 24(3): 120-126.

Keywords: Vitamin D, Alzheimer's disease, mild cognitive impairment, progression

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Introduction

Alzheimer's disease (AD) is the commonest cause of a cognitive deterioration in older age. Moreover, the incidence of AD increases with age; the median age of diagnosis is approximately 80 years of age[1]. Therefore, the prevalence is growing due to the aging of the population[2]. Considering that it is an incurable neurodegenerative disease, many researchers are searching for preventive interventions to delay the development of dementia.

In recent years, investigators have acknowledged vitamin D deficiency as a risk factor influencing skeletal and brain health and promoting neurocognitive impairment[3]. Many potential mechanisms have been identified which associate low vitamin D levels with the risk of dementia[4]. Latest studies proved that vitamin D plays an essential role in neuroprotection, neurotransmission, and neurotrophy[5]. Moreover, experimental studies have demonstrated the association of vitamin D with a higher clearance of amyloid β[4].

Previous systematic reviews and meta-analyses from cross-sectional studies observed that deficiency of vitamin D is correlated with the risk of developing dementia and AD[6,7]. Moreover, longitudinal population-based studies have found that vitamin D deficiency may increase the risk of cognitive deterioration and AD over time[8,9], although conflicting results exist as well[10]. Additionally, numerous reports suggest a relationship between vitamin D deficiency with mild cognitive impairment (MCI) and

AD[11-13].

MCI is identified as a clinical stage in-between the anticipated cognitive loss associated with normal aging and the earliest features of dementia[14]. It has been reported that 10 %-20 % of individuals over 65 suffer from MCI[14] with a high potential to convert to AD[15,16].

This study aimed to investigate the association between serum 25(OH)D levels, which is considered a stable marker regarding vitamin D status, with MCI and AD older Greek adults. In addition, it investigated whether vitamin D deficiency could be considered a predisposing factor for MCI individuals developing dementia.

Methods

A longitudinal cross-sectional study was conducted at the University General Hospital of Alexandroupolis, which included 350 consecutive eligible subjects aged 65 old and over. The participants were allocated into three groups consisting of 103 healthy subjects (HS), 109 individuals with MCI, and 138 patients with AD. The groups of MCI and AD were recruited between January 2012 and December 2015 among the consecutive eligible individuals examined in the dementia outpatient clinic. The third group included 103 well-functioning volunteer subjects among the relatives of the patients attending the dementia outpatient clinic, who fulfilled the study's terms. All groups were gender-balanced and aged-matched. One follow-up examination was performed among the MCI individuals 30 months ± three months after the initial evaluation.

Data collection included: date of evaluation, demographic data, anthropometrical data (body weight in Kg, height in m), education level, medical history, regular medication use, sunscreen use, neurological examination, neuropsychological testing, blood pressure, and blood sampling. In addition, all the subjects diagnosed with MCI and AD, according to the policy of the outpatient dementia clinic, had a brain magnetic resonance imaging (MRI) examination [or brain computed tomography (CT) if there was a contraindication to perform MRI). The authors of the study completed data collection, and variability was checked.

According to eligibility criteria, we included patients older than 65 years and fluent in Greek. Exclusion criteria comprised the presence of other neurological or psychiatric disorders, severe ophthalmological or acoustic diseases that could affect the prosecution of the neuropsychological assessments, and reversible causes of dementia such as severe hypothyroidism or vitamin B12 deficiency. In addition, we excluded from the study individuals with a known history of osteoporosis and/or use of vitamin D supplements and bisphosphonates, primary hyperparathyroidism, malabsorption syndrome, chronic kidney disease, tuberculosis, HIV, active granulomatous disease, and use of anticonvulsants medications.

Assuming a serum 25-hydroxyvitamin D [25(OH)D] concentration in older healthy subjects at [mean ± standard deviation (SD)] 26.16 ± 8.73 ng/ml[17], it was calculated (http://www.openepi.com/Menu/OE_Menu.htm) that a sample size of at least 85 individuals in each group was required to detect a clinically significant difference of 25(OH)D values of 0.5 SD, with the level of significance at a =0.05 and statistical power of 90 %.

We selected a sample of 456 participants (155 HS, 155 MCI, 156 AD) aged 65 years and over, out of whom we excluded 35 subjects (5 HS, 12 MCI, 18 AD) who had, according to medical records, a previous predominant cardiovascular disease or stroke (defined as one or more of the following: atrial fibrillation, implantable cardioverter defibrillator, pacemaker, congestive heart failure, coronary heart disease, claudication, stroke, or transient ischemic attack)[8]. We also excluded 13 subjects (7 HS, 6 MCI) with inadequate samples to serum vitamin D levels and 64 participants (40 HS, 24 MCI) with missing data for covariates, while four participants did not show up on the follow-up. All the study's participants were ambulatory, and none used sunscreen.

This study was performed according to the ethical standards of the 1964 Helsinki Declaration and its later amendments; was approved by the Ethical Committee of the University Hospital of Alexandroupolis approved the study (No 9, date: 12/12/2012), and informed written consent was obtained from all participants or their relatives before participating in the study.

The diagnosis of MCI was performed according to Petersen's criteria[14], while AD diagnosis was according to recommendations of the National Institute of Neurologic and Communicative Disorders and Stroke and AD and Related Disorders Association (NINCDS-ADRDA). The cognitive evaluation included the validated in Greek Mini-Mental State Examination (MMSE), according to each subject's educational level[18] and the Greek version of CAMCOG[19]. The HS group included subjects without a memory complaint and according to neurocognitive test results, adjusted for age and educational level.

Peripheral blood was collected from each participant, centrifuged at 400 x g for 20 min, and the serum stored at -80 ºC. We measured the serum 25(OH)D levels in 2018 utilizing an electrochemiluminescence binding assay for the in-vitro assessment of total 25(OH)D (Elecsys Vitamin D Assay, Roche Diagnostics International Ltd., Rotkreuz, Switzerland). This method was standardized using internal standards traceable to the isotope dilution liquid chromatography/tandem mass spectrometry (ID LC MS/MS) 25(OH)D reference measurement procedure[20].

It is still controversial which vitamin D levels are considered normal and adequate[21,22]. Therefore, in our study, we examined the serum levels of 25(OH)D, and participants were classified, based on 25(OH)D value, into three groups, severe deficiency (<10 ng/ml), moderate deficiency (10-20 ng/ml), and adequate vitamin D supply (>20 ng/m)[7,22].

We adjusted for covariates that were considered possible confounders: age expressed in years, the season of blood collection (Winter-Spring/Summer-Autumn), educational status (low: did not finish elementary school,

medium: finished elementary school/some secondary school, high: completed high school), sex, body mass index (BMI; kg/m^2), and presence of vascular risk factors included hypertension (≥140 or 90 mmHg for systolic or diastolic blood pressure respectively, or use of antihypertensive drugs) and/or diabetes (fasting serum glucose of ≥126 mg/dL or use of insulin or oral hypoglycemic agents, according to the Hellenic Diabetes Association guidelines, 2017).

Statistical analysis

We analyzed data with the IBM SPSS Statistics for Windows, Version 19.0 (IBM Corp., Armonk, NY, USA). We used the Kolmogorov-Smirnov test to determine the normality of quantitative variables. The qualitative variables are reported as frequencies and percentages (%), while the quantitative variables are presented as mean values ± standard deviation (SD). Regarding demographics, clinical characteristics, and serum 25(OH)D concentrations, to assess differences between HS, individuals with MCI, and patients with AD, the Student's t-test, chi-square test, and analysis of variance (ANOVA) were used. Pearson's r correlation coefficient was used to assess the linear relation between serum 25(OH)D concentrations and the subjects' quantitative characteristics. Receiver operating characteristic (ROC) analysis was utilized to evaluate the diagnostic significance of serum 25(OH)D concentrations. The area under the ROC curve (AUC), sensitivity, specificity, positive and negative predictive values were calculated, and the Cohen's kappa was used to assess agreement. The optimal cut-off values were derived according to the Youden Index. Multivariable logistic regression models were used to calculate odds ratios (OR) and 95% confidence intervals (CI) as the measure of the association between serum 25(OH)D concentrations and the prevalence of MCI, AD, and the MCI conversion to AD, controlling for age, the season

of blood collection, educational status, sex, BMI, and the presence of vascular risk factors included hypertension or/and diabetes. All tests were two-tailed, and p values <0.05 were considered statistically significant.

Results

The demographic and clinical characteristics of HS, individuals with MCI, and patients with AD are presented in Table 1. We did not find any statistically significant differences regarding gender, age, BMI, vascular risk factors and seasonal collection of blood between the groups. The scores of CAMCOG and MMSE were statistically decreased in MCI and AD patients compared to HS, and in AD compared to MCI patients. In contrast to HS, serum 25(OH)D concentrations were significantly lower in patients with AD (p <0.001) and in individuals with MCI (p =0.012) (Table 2). Controlling for the effect of all possible confounders, multivariate analysis revealed that an increase of vitamin D concentration by one ng/mL reduces the risk of MCI by 4 % [adjusted odds ratio (aOR) =0.96, 95 % confidence interval (CI) =0.92-0.99, p =0.006] and the risk of AD by 8 % (aOR =0.92, 95 % CI =0.89-0.95, p <0.001). The results of the ROC analysis revealed that the optimal cut-off points for the discrimination of MCI and AD patients from HS were 18.35 ng/ml and 13.95 ng/ml, respectively, which yielded moderate sensitivities (70.6 % and 62.3 %, respectively) and specificities (51.5 % and 68.9 %, respectively) (Table 3).

When compared to individuals with MCI, serum 25(OH)D concentrations were considerably lower in AD patients (p =0.003) (Table 2). After adjusting for potential confounders, multivariate analysis revealed that an increase of vitamin D concentration by one ng/mL in an individual with MCI reduces the risk of progression to AD by 5 % (aOR =0.95, 95 % CI =0.92-0.98, p =0.003). Based on the ROC curve, the optimal cut-off point for the discrimination of AD from MCI patients was 11.10

Table 1: The demographic and clinical characteristics of healthy subjects, individuals with mild cognitive impairment and Alzheimer's disease who were included in the study.

Characteristic	HS	MCI	AD	HS vs MCI*	HS vs AD*	MCI vs AD*
Number	103	109	138			
Male gender	50 (48.5)	46 (42.2)	61 (44.2)	0.354	0.504	0.753
Age (years)	74.00 ± 5.41	74.52 ± 5.48	75.51 ± 6.72	0.488	0.214	0.062
BMI (kg/m^2)	27.87 ± 2.21	27.74 ± 2.84	27.21 ± 3.09	0.697	0.394	0.168
Education level				0.572	0.132	0.122
Low	15 (14.6)	19 (17.4)	34 (24.6)			
Medium	52 (50.5)	59 (54.1)	57 (41.3)			
High	36 (35.0)	31 (28.4)	47 (34.1)			
VRF (%)	26 (25.2)	27 (24.8)	34 (24.6)	0.937	0.914	0.981
CAMGOG	84.23 ± 9.21	80.69 ± 12.19	64.36 ± 16.98	0.018	<0.001	<0.001
Season tested 25(OH)D				0.222	0.169	0.930
Winter-Spring	49 (47.6)	61 (56.0)	78 (56.5)			
Summer-Autumn	54 (52.4)	48 (44.0)	60 (43.5)			
MMSE	27.88 ± 1.54	26.03 ± 2.14	16.36 ± 5.51	<0.001	<0.001	<0.001

Values are given as numbers and percentage in brackets or mean ± standard deviation, HS: healthy subjects, MCI: individuals with mild cognitive impairment, AD: individuals with Alzheimer's disease, *: p values, BMI: body mass index, VRF: vascular risk factors, CAMCOG: Cambridge Cognition Examination, 25(OH)D: 25-hydroxyvitamin D, MMSE: Mini Mental State Examination.

Table 2: Serum 25-hydroxyvitamin D concentrations in healthy subjects, individuals with mild cognitive impairment, Alzheimer's disease, individuals with mild cognitive impairment after the follow-up examination, and individuals with mild cognitive impairment who progressed to Alzheimer's disease after the follow-up examination.

	HS	MCI	p value	aOR (95 CI)	p value
25(OH)D (ng/mL)	18.97 ± 8.34	16.00 ± 8.81	0.012	0.96 (0.92-0.99)	0.006
25(OH)D, n (%)			0.015		
<10 ng/mL	18 (17.5)	32 (29.4)		3.22 (1.46-7.12)	0.004
10 – 20 ng/mL	45 (43.7)	53 (48.6)		2.02 (1.05-3.87)	0.034
>20 ng/mL	40 (38.8)	24 (22.0)		Reference	
	HS	AD	p value	aOR (95 CI)	p value
25(OH)D (ng/mL)	18.97 ± 8.34	12.88 ± 7.68	<0.001	0.92 (0.89-0.95)	<0.001
25(OH)D, n (%)			<0.001		
<10 ng/mL	18 (17.5)	62 (44.9)		4.96 (2.37-10.39)	<0.001
10 – 20 ng/mL	45 (43.7)	52 (37.7)		1.99 (1.05-3.78)	0.036
>20 ng/mL	40 (38.8)	24 (17.4)		Reference	
	MCI	AD	p value	aOR (95 CI)	p value
25(OH)D (ng/mL)	16.00 ± 8.81	12.88 ± 7.68	0.003	0.95 (0.92-0.98)	0.003
25(OH)D, n (%)			0.044		
<10 ng/mL	32 (29.4)	62 (44.9)		1.98 (0.98-4.07)	0.062
10 – 20 ng/mL	53 (48.6)	52 (37.7)		1.00 (0.50-1.99)	0.998
>20 ng/mL	24 (22.0)	24 (17.4)		Reference	
	MCI remained	MCI to AD	p value	aOR (95 CI)	p value
25(OH)D (ng/mL)	17.62 ± 6.84	14.62 ± 6.92	0.028	0.90 (0.83-0.96)	0.003
25(OH)D, n (%)			0.032		
<10 ng/mL	10 (23.3)	22 (33.3)		6.11 (1.76-21.23)	0.004
10 – 20 ng/mL	18 (41.9)	35 (53.0)		4.56 (1.51-13.79)	0.007
>20 ng/mL	15 (34.9)	9 (13.7)		Reference	

Values are given as numbers and percentage in brackets or mean ± standard deviation, 25(OH)D: 25-hydroxyvitamin D, HS: healthy subjects, MCI: individuals with mild cognitive impairment, AD: individuals with Alzheimer's disease, remained MCI: individuals with mild cognitive impairment after the follow-up examination, MCI to AD: individuals with mild cognitive impairment who progressed to Alzheimer's disease after the follow-up examination, 95 CI: 95 % confidence interval, aOR: adjusted odds ratio for age, season of blood collection, educational status, sex, body mass index (BMI) and the presence of vascular risk factors included hypertension or/and diabetes.

Table 3: Receiver operating characteristic analysis for the evaluation of the diagnostic significance of serum 25-hydroxyvitamin D concentrations for (i) mild cognitive impairment and (ii) Alzheimer's disease.

	(i) HS vs MCI	p value	(ii) HS vs AD	p value
AUC (95 % CI)	0.598 (0.522 - 0.675)	0.013	0.696 (0.630 - 0.762)	<0.001
Cut-off	≤ 18.35		≤ 13.95	
Sensitivity (%)	70.6 (61.2 - 79.0)		62.3 (53.7 - 70.4)	
Specificity (%)	51.5 (41.4 - 61.4)		68.9 (59.1 - 77.7)	
PPV (%)	60.6 (55.0 - 66.0)		72.9 (66.2 - 78.7)	
NPV (%)	62.4 (54.0 - 70.1)		57.7 (51.5 - 63.7)	
Overall agreement (%)	61.3 (54.4 - 67.9)		65.2 (58.8 - 71.2)	
Cohen's kappa	0.222	0.001	0.305	<0.001
OR (95 % CI)	2.55 (1.45 - 4.49)	0.001	3.67 (2.14 - 6.30)	<0.001

HS: healthy subjects, MCI: individuals with mild cognitive impairment, AD: individuals with Alzheimer's disease, AUC: area under the curve, CI: confidence interval, PPV: positive predictive value, NPV: negative predictive value, OR: odds ratio.

ng/ml, which yielded a low sensitivity of 50.0 % and a moderate specificity of 71.6 % (Table 4).

Sixty-six (60.6 %) patients with MCI converted to AD during the follow-up time. Patients with MCI who converted to AD had significantly decreased serum 25(OH)D concentrations when compared to patients who remained with MCI (p =0.028) (Table 2). After adjusting for potential confounders, multivariate analysis revealed an increase of vitamin D concentration by one ng/mL in an individual with MCI reduces the risk of conversion to AD by 10 % (aOR =0.90, 95 % CI =0.83-0.96, p =0.003). Multivariate logistic regression analysis also showed that independent determinants for conversion from MCI to AD were male gender (p =0.020), MMSE (p <0.001), CAMGOG (p =0.024), as well as medium (p =0.046) and high (p =0.076) education level (Table 5). Based on the ROC curve, the optimal cut-off point for the discrimination of patients with MCI who progressed or not to AD

was 14.90 ng/ml. This resulted in low sensitivity of 57.6 % and a moderate specificity of 67.4 % (Table 4).

Additionally, vitamin D deficiency (<20ng/ml) was more frequent in MCI than HS (p=0.015), in AD than HS (p<0.001), in AD than MCI (p=0.003), and in individuals with MCI who converted to AD compared to those who remained MCI (p=0.032). In particular, adjusting for all potential confounders, multivariate logistic regression analysis revealed that severe 25(OH)D deficiency (<10 ng/ml) was associated with a 3-fold increase in the odds of MCI (aOR =3.22, p =0.004), a 5-fold increase in the odds of AD (aOR =4.96, p <0.001) and a 6-fold increase in the odds of conversion from MCI to AD (aOR =6.11, p =0.007) (Table 2).

Serum 25(OH)D concentrations were higher in the samples collected during summer or autumn, according to the correlation of serum concentrations of 25(OH)D with participants' characteristics. However, this difference reached statistical significance among HS (p <0.001) and AD patients (p =0.002) but not in individuals with MCI (p =0.423). Furthermore, serum concentrations of 25(OH)D were negatively correlated (r =-0.229, p =0.020) with age among HS. No other statistically significant associations were found.

Discussion

In our study, patients with MCI and AD demonstrated significantly lower serum concentrations of 25(OH)D HS. In particular, an increase of vitamin D concentration by one ng/mL reduces the risk of MCI by 4 % and the risk of AD by 8 %. These results confirm recent data from systematic reviews and meta-analysis from case-control, cross-sectional, and cohort studies that found serum levels of 25(OH)D to be inversely related to the risk of dementia and AD, although reverse causality remains a possibility[6,7].

Moreover, in the longitudinal component of the current study, we observed negative relation between concentrations of 25(OH)D and the risk of incidence AD among the MCI population. This association remained robust after adjustment for a variety of potential confounders. In particular, an increase of vitamin D concentration by one ng/mL reduces the risk of conversion to AD by 10 %. To date, there is insufficient data to investigate the association between concentrations of vitamin D in MCI individuals and the risk of AD.

However, according to two extensive previous prospective population-based studies for dementia with long-term follow-up, an association between vitamin D deficiency and an enhanced risk for all-cause dementia

Table 4: Receiver operating characteristic analysis for the evaluation of the discrimination ability of serum 25-hydroxyvitamin D concentrations of (i) mild cognitive impairment from Alzheimer's disease and (ii) mild cognitive impairment progressed or not to Alzheimer's disease.

	(i) MCI vs AD	p value	(ii) MCI remained vs MCI conversed to AD	p value
AUC (95 % CI)	0.601 (0.530 - 0.672)	0.007	0.631 (0.522 - 0.739)	0.022
Cut-off	≤ 11.10		≤ 14.90	
Sensitivity (%)	50.0 (41.4 - 58.6)		57.6 (44.8 - 69.7)	
Specificity (%)	71.6 (62.1 - 79.8)		67.4 (51.5 - 80.9)	
PPV (%)	69.0 (61.3 - 75.8)		73.1 (62.7 - 81.4)	
NPV (%)	53.1 (48.0 - 58.1)		50.9 (42.2 - 59.5)	
Overall agreement (%)	59.5 (53.1 - 65.7)		61.5 (51.7 - 70.6)	
Cohen's kappa	0.208	0.001	0.237	0.011
OR (95 % CI)	2.52 (1.48 - 4.29)	0.001	2.81 (1.26 - 6.28)	0.011

MCI: individuals with mild cognitive impairment, AD: individuals with Alzheimer's disease, AUC: area under the curve, CI: confidence interval, PPV: positive predictive value, NPV: negative predictive value, OR: odds ratio.

Table 5: Results of multivariate logistic regression analysis for the prediction of the progression from mild cognitive impairment to Alzheimer's disease.

	b Coefficient	Standard error	aOR	95 % CI	p value
Constant	24.395	5.206			
Male gender	1.356	0.581	3.88	1.24-12.12	0.020
MMSE	-0.682	0.178	0.51	0.36-0.72	<0.001
CAMGOG	-0.057	0.025	0.95	0.90-0.99	0.024
Education level					
Low					
Medium	-1.682	0.843	0.19	0.04-0.97	0.046
High	-1.600	0.902	0.20	0.03-1.18	0.076
25(OH)D	-0.112	0.033	0.90	0.83-0.96	0.003

MCI: individuals with mild cognitive impairment, AD: individuals with Alzheimer's disease, aOR: adjusted odds ratio, CI: confidence interval, MMSE: Mini Mental State Examination, CAMCOG: Cambridge Cognition Examination, 25(OH)D: 25-hydroxyvitamin D.

and AD in older persons has been established[8,9]. These studies included older cognitive healthy participants during a different follow-up time ranged from 5.6 to 13 years. However, there are mixed results when it comes to cognitive impairment over time[10]. Furthermore, this study revealed that severe deficiency of 25(OH)D (<10 ng/ml) was associated with an increase in the odds of MCI (3-fold), AD (5-fold), and conversion from MCI to AD (6-fold increase). Recent data have also indicated that the risk of dementia and AD was increased with decreased vitamin D[7].

The diagnostic significance of 25(OH)D for MCI and AD was moderate in the present study. Although recent studies have used vitamin D as a possible biomarker for cognitive dysfunction and AD[11,23], our results cannot support this relation, as the sensitivity and the specificity from ROC analysis were not greater than 85 %.

According to our results, sixty-six (60.6%) individuals with MCI progressed to AD during 30 months ± three months of follow-up time. In contrast, reported annual rates of progression from MCI to dementia have ranged from five to 17 %, with lower progression rates observed in population-based studies and higher rates in clinical centers and memory clinics[15,16]. Moreover, in our study, we excluded individuals with factors that might increase the risk of dementia.

Multivariate logistic regression analysis showed that independent determinants for conversion from MCI to AD were male gender, MMSE, CAMGOG, and medium and high education level. Nevertheless, in previous studies, gender was not found to be a predictor[15]. The MMSE remains the most thoroughly studied instrument[24]. It is also essential to assess predicting conversion from MCI to dementia combined with other tests rather than alone[25]. CAMCOG is part of the CAMDEX (The Cambridge Mental Disorders of the Elderly Examination) and has shown good accuracy in predicting AD in individuals with low educational level[26].

According to the findings of our study, serum concentrations of 25(OH)D were higher in the samples obtained throughout summer or autumn. Vitamin D levels have been reported to rise in the summer and lower in winter[27] due to the dependency of vitamin D on sunlight. This seasonal variation may, according to previous studies[28], be affected by latitude. Nevertheless, vitamin D deficiency has been recorded even in sunny regions[17]. In a recent Greek study regarding healthy Greek men and women, vitamin D deficiency was quite prevalent, with the winter-spring season of sampling to be also a negative determinant[29]. What is more, a negative correlation between serum 25(OH)D concentrations and age among HS has been found. Limited exposure to the sun, decreased skin synthesis of pre-vitamin D, and reduced hydroxylation in the kidney associated with the aging process explain the high prevalence of vitamin D deficiency in older adults[12].

Our study has several methodological limitations. First, hypovitaminosis D is a usual condition among the elderly[12]. However, when vitamin D concentrations were considered a continuous variable, we found a link between 25(OH)D levels and MCI and AD. Assessment of 25(OH) D was only conducted at the initial evaluation, although this fact agrees with previous analyses, which reported that a single measurement is a reliable indicator of long-term plasma 25(OH)D exposure[30].

Additionally, in the initial design of the study, we considered three groups, HS, MCI, and AD. In the sequence, MCI patients were followed prospectively to assess the impact of 25(OH)D levels on the conversion to AD, considering all other MCI group characteristics. Moreover, we did not take into consideration all the known factors that influence circulating 25(OH)D levels, such as genetic variants in the vitamin D receptor gene (VDR gene)[4]. Furthermore, we did not identify sun exposure, skin phenotype, and dietary vitamin D intake as potential confounders. Recent data suggest that the impact of these factors in vitamin D deficiency is still under investigation[31].

Moreover, although the authors established MCI and AD diagnosis, a degree of misclassification cannot be totally avoided since many AD cases may constitute a mixture of pathologies. Finally, our findings cannot in themselves demonstrate a causative connection between vitamin D deficiency and cognitive decline as there is the possibility of immeasurable confounders.

On the other hand, our study has significant strengths. A team of expert neurologists evaluated all subjects according to the international criteria (NINCDS-ADRDA). Furthermore, the present study contains a longitudinal component that enables the investigators to take confounding factors into account and study the cause and effect's temporal order.

Conclusions

The present study in older Greek adults revealed that serum levels of vitamin D are significantly lower in individuals with MCI and AD patients than HS and significantly lower in patients with MCI who progressed to AD than those who remained MCI. These results may suggest that maintaining adequate serum levels of vitamin D in elderly subjects could contribute to delay or prevent cognitive deterioration.

Conflict of interest

The authors declare no conflicts of interest.

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