

VITAMIN D IMPACT ON STRESS AND COGNITIVE DECLINE IN OLDER ROMANIAN ADULTS

JUSTIN AURELIAN¹, ANDREEA ZAMFIRESCU², MIRELA NEDELESCU^{3*}, SMARANDA STOLERU⁴, SANDRA MONICA GÎDEI², COSTINA DANIELA GÎȚĂ², ANA PRADA², CORINA OANCEA⁵, ANDREEA-IULIA VLADULESCU-TRANDAFIR⁶, SORINA MARIA AURELIAN²

¹"Carol Davila" University of Medicine and Pharmacy, Department of Nursing, Bucharest, Romania

²"Carol Davila" University of Medicine and Pharmacy, Clinic of Geriatrics, Hospital of Chronic Diseases "Sf. Luca", Bucharest, Romania

³"Carol Davila" University of Medicine and Pharmacy, Department of Hygiene and Medical Ecology, Bucharest, Romania

⁴"Carol Davila" University of Medicine and Pharmacy, Discipline of Pharmacology and Pharmacotherapy, Bucharest, Romania

⁵"Carol Davila" University of Medicine and Pharmacy, Department of Physical Medicine and Rehabilitation, Bucharest, Romania

⁶"Carol Davila" University of Medicine and Pharmacy, Department of Rheumatology and Physical Medicine and Rehabilitation, Bucharest, Romania

*corresponding author: mirela.nedelescu@umfcd.ro

Manuscript received: October 2024

Abstract

Vitamin D plays a key role in ageing-related cellular processes, such as inflammation, oxidative stress and mitochondrial dysfunction. Vitamin D deficiency is prevalent among older adults and has been linked to cognitive decline, mood disorders and various age-related diseases. This study evaluated the correlation between serum vitamin D levels and stress resilience, cognitive decline and successful ageing in older Romanian adults. A cross-sectional clinical trial was conducted with 89 participants over 55. Serum vitamin D levels were measured using chemiluminescence analysis. At the same time, the cognitive function was assessed through the Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MOCA) and the Geriatric Depression Scale. Stress and resilience were evaluated using the Perceived Stress Scale and Siebert Resilience Test. Low vitamin D levels have been associated with cognitive impairment, as measured by the MMSE and MOCA. However, no significant relationship was found between vitamin D levels and stress or resilience scores. This study indicates that vitamin D may play a significant role in preventing cognitive decline among older adults; however, its effects on stress and resilience necessitate further investigation.

Rezumat

Vitamina D joacă un rol important în procesele celulare asociate cu îmbătrânirea, cum ar fi inflamația, stresul oxidativ și disfuncția mitocondrială. Carența de vitamină D este răspândită în rândul adulților în vârstă și a fost legată de deficitul cognitiv, tulburările de dispoziție și alte boli ale vârstnicului. Acest studiu și-a propus să evalueze corelația dintre nivelurile serice de vitamină D și rezistența la stres, declinul cognitiv și îmbătrânirea cu succes la vârstnicii din România. Un studiu clinic transversal a fost efectuat cu 89 de participanți având vârsta peste 55 de ani. Nivelurile serice de vitamină D au fost măsurate folosind analiza de chemiluminiscență, în timp ce funcția cognitivă a fost evaluată prin *Mini-Mental State Examination* (MMSE), *Montreal Cognitive Assessment* (MOCA) și *Geriatric Depression Scale*. Stresul și reziliența au fost evaluate utilizând Scala de stres perceput și Testul de reziliență psihică Siebert. A fost identificată o corelație semnificativă între nivelurile scăzute de vitamină D și afectarea cognitivă, măsurată de MMSE și MOCA. Cu toate acestea, nu a fost găsită nicio relație semnificativă între nivelurile de vitamină D și scorurile de stres sau rezistență. Acest studiu sugerează că vitamina D joacă un rol în prevenirea declinului cognitiv la vârstnici, dar impactul său asupra stresului și rezilienței psihice necesită investigații suplimentare.

Keywords: Vitamin D, cognitive decline, stress resilience, ageing, Romanian adults, clinical trial

Introduction

According to growing evidence, numerous cellular processes, such as autophagy, mitochondrial dysfunction, inflammation, oxidative stress, epigenetic changes, DNA perturbations and changes in complex cellular signalling mechanisms mediated by calcium ions and reactive oxygen species (ROS), are causing ageing. What

distinguishes all of these cellular ageing mechanisms is that vitamin D regulates their activity [2].

Hypovitaminosis D is ubiquitous; however, it is especially prevalent in older individuals. Most non-bone tissues exhibit non-classical vitamin D responses based on serial doses of 25-hydroxyvitamin D (25(OH)D). Hypovitaminosis D is related to an increased risk of

sepsis (bacterial, mycobacterial and viral) and cardiovascular and metabolic disorders (*e.g.*, hyperlipidaemia, type 2 diabetes, acute vascular events, dementia, stroke, or heart failure) [9]. Many types of cancer are associated with vitamin D deficiency, although causality is only accepted for colorectal cancer. Vitamin D activates receptors on neurons involved in behavioural regulation, stimulates neurotrophin release and protects the brain by modulating the defence against vascular damage with antioxidant and anti-inflammatory compounds and improving metabolic and cardiovascular functions. This particular vitamin plays a significant role in innate immunity by activating Toll-like receptors and reducing cytokine production. Additionally, it is involved in adaptive immunity, where it regulates the production of T lymphocytes and inhibits interferon-gamma production – both critical pathways associated with autoimmune and infectious diseases. The importance of this vitamin was extensively researched during the Coronavirus Disease 2019 (COVID-19) pandemic [48]. Elevated parathyroid hormone levels have also been linked to depression, weariness and disorientation, which appears to be due to poor calcium absorption. More research is needed to determine the effect of vitamin D supplementation on cognitive impairment and mood disorders [7, 15]. Vitamin D deficiency has been linked to cognitive impairment. It has been involved in the development of many types of dementia, including Alzheimer's disease (which is responsible for up to 80% of dementia cases) [3]. Additionally, vitamin D may have preventive effects against cognitive loss since it contributes to psychological resilience - the capacity to retain mental stability under stress [23, 47]. Resilience is the ability to adapt to hardship, often measured by well-being following significant life changes. Resilience is the capacity to adapt to adversity and is frequently assessed by the level of well-being experienced following significant life changes. Alternatively, resilience has been conceptualized as a psychological trait that facilitates adaptation through consistent individual characteristics. These perspectives may be interconnected with the individual differences observed in regulating stress in daily situations [23]. According to the World Health Organisation (WHO), stress is a condition of worry or mental tension caused by a challenging circumstance to overcome [51]. Nowadays, stress is defined as a set of experiences that elicit annoyance or anxiety due to a perceived threat to one's security or the individual's inadequate ability to manage such circumstances. As a result, addressing modifiable risk factors, including food habits and other behavioural factors such as tobacco use, may help manage perceived individual stress levels. Several investigations have found that low vitamin 25(OH)D levels are linked to a variety of health-threatening diseases, including breast cancer [25], cardio-metabolic problems [4, 11, 49], musculoskeletal

pathologies [3, 31], depression [30] and sleep disturbances [19].

Low vitamin D levels may also cause weakness and degradation of glycolytic muscle fibres. The current vitamin D intake in Europe is insufficient, resulting in widespread deficiency among the general population. This situation represents a significant public health concern. Given that vitamin D supplementation can improve symptoms of melancholy and anxiety [47], investigating its levels in the general population and promoting proper intake through nutrition is critical for attempting to manage stress from several perspectives. This study aims to better understand the effect of vitamin D status on perceived stress, cognitive decline and resilience in an older Romanian population. We also investigated whether vitamin D can be considered a marker for successful ageing or a predictor of early dementia status.

Materials and Methods

Study design

We conducted a cross-sectional clinical trial to evaluate the correlation between serum levels of vitamin D (25-OH-vitamin D) and stress, resilience and cognitive decline in older adults.

The study comprised 100 patients enrolled from May to September 2024 in the Geriatrics and Gerontology Clinic at St. Luke Hospital Bucharest, Romania. The Ethical Review Board of St. Luke Hospital Bucharest, Romania, approved the study protocol, which was conducted according to the principles of Declaration of Helsinki.

Inclusion criteria. Eligible patients were men and women over 55 years old and negative for COVID-19.

Exclusion criteria. Participants with severe depression, mental illness, cancer of any type and history of any severe disease diagnosis (including severe diabetes, cardiovascular disease, *etc.*). The study excluded 9 individuals who had severe Alzheimer's Disease. We excluded those patients because we also inquired about their stress levels and resilience. Patients suffering from severe dementia or depression struggled and could not accurately complete these complex questionnaires. Other 2 eligible individuals were also excluded because they had refused to join the study, so 89 participants participated in this randomised study at the beginning. The patients who consumed dietary supplements or vitamin D medications in the past three months were also excluded.

Participants

We included 89 participants for further analysis, all meeting the eligibility criteria and providing written, informed consent before the study. Of the participants, 14 were male and 75 were female, with a mean age of 70.8 ± 8.53 .

The primary outcome was the patient's global assessment of vitamin D level. Other outcome measurements included cognitive status, MMSE, MOCA, Clock-drawing test, Geriatric Depression Scale Short Form and demographic characteristics. For "brain successful ageing," it was important to evaluate participants through stress and resilience scales.

Evaluation tools and biochemical assay

Biochemical assay

A qualified nurse obtained venous blood samples to assess the concentration of 25-hydroxyvitamin D, glucose and cholesterol. We measured the vitamin D blood level on the second day of admission, in the morning after overnight fasting. The serum 25-hydroxyvitamin D [25(OH)D] was determined using chemiluminescence analysis by the Architect i1000. The following categories of Vitamin D status were defined: severe deficiency – lower than 10 ng/mL, moderate deficiency – between 10 - 20 ng/mL, low deficiency – 20 - 29.9 ng/mL and sufficiency \geq 30 ng/mL. Cognitive status assessment

The MMSE was administered to assess the participants' cognitive function. Scores range from 0 to 30, with higher scores indicating better cognitive functioning. Based on the test design, a score of 21 - 26 points was used to indicate cognitive impairment [50].

MOCA is a concise screening tool for moderate cognitive impairment (MCI). The Alzheimer's Society recommends using it to measure cognitive symptoms objectively in clinical settings. The MOCA test is valid in multiple languages, although moderately so in a Romanian-language geriatric memory clinical setting [16].

The Geriatric Depression Scale-15 (GDS-15) is a 15-item variation of the GDS with solid reliability and consists of 15 dichotomous questions (yes or no). Scores range from 0 to 15. A score of 6 or higher, out of a maximum of 15 points, is a potential indicator of depression [42].

The clock-drawing test (CDT) is a test used to screen cognitive dysfunction secondary to dementia, including Alzheimer's disease [1].

Stress and Resilience Measurement

Stress levels were assessed using the Perceived Stress Scale (PSS) by Levenstein, a validated tool measuring stress perception in daily life. The PSS consists of 30 items scored on a 5-point Likert scale. The score with limits between 30 and 120 points allows placing the subject in one of the 3 categories: low stress, moderate stress and stress intensive [29]. Resilience was measured using the Siebert Resilience test, a 20-item questionnaire that evaluates the ability to bounce back from adversity. Each item is rated on a 1 to 5-point Likert scale, from "very little true" to "true nearly all the time". The total score ranges from 50 to 90 points, with higher scores indicating greater resilience [9].

Procedure

Following the blood draw, participants completed the stress and resilience questionnaires in a quiet environment to minimise distractions, with assistance provided for any participant requiring help with reading or comprehension. The MMSE, GDS and CDT were administered by trained personnel, following standard procedures. The test took approximately 10 - 15 min *per* participant. Demographic data, including age, gender, education level, smoking habits and medical history (*e.g.*, chronic illnesses or medication affecting vitamin D metabolism), were also systematically collected from patient charts.

Statistical Analysis

All the data from this study were analysed using IBM SPSS Statistics 25 and illustrated using Microsoft Office Excel/Word 2021. Descriptive statistics (means, standard deviations, frequencies) were computed for demographic variables, vitamin D levels and cognitive scores. Quantitative variables were tested for normal distribution using the Shapiro–Wilk test and were written as averages with standard deviations or medians with interquartile ranges. Student's t-test was used for parametric distributions. Quantitative variables were tested between measurements using Friedman's tests along with Dunn–Bonferroni post hoc tests. Quantitative independent variables were tested between groups using Mann–Whitney U tests, and their correlations were calculated using Spearman's rho correlation coefficient. The qualitative variables were written as absolute frequencies with percentages and were tested between groups using Fisher's Exact Test.

Additionally, multiple regression analyses were performed to assess the independent effects of vitamin D levels and cognitive scores, controlling for potential confounders such as age, gender and level of studies. A p-value of < 0.05 was considered statistically significant. One-way analysis of variance (ANOVA) and Tukey HSD tests were applied to demonstrate correlations between vitamin D level and cognitive function expressed through stress and resilience assessment.

Results and Discussion

The study cohort consisted of 89 selected patients based on established inclusion and exclusion criteria. Among these participants, 75 were female and 14 were male. Their higher life expectancy can justify the higher percentage of women. The average age of the patients was 70.08 ± 8.53 years. Patients were classified into age groups: adults (< 65 years), elderly (65 - 74 years), very elderly (> 85 years) and long-lived subjects (> 85 years). The study group includes, in approximately equal proportions, married and unmarried individuals with good financial status and average educational level, most of them being smokers, as seen in Table I. Table II presents statistical data

on the parameters extracted from the patients (blood parameters, results of applied cognitive test).

The mean vitamin D level was 24.03 ± 10.105 ng/ml, resulting in the following categories: 1) severe vitamin D deficiency: vitamin D level lower than 10 ng/mL in the blood (3.37% - 3 participants); 2) moderate vitamin D deficiency: vitamin D blood levels between 10 - 19.9 ng/mL (35.95% - 32 participants); 3) vitamin D relative insufficiency: vitamin D levels between 20 - 29.9 ng/mL (38.2% - 34 participants); 4) adequate level of vitamin D: blood vitamin D level greater than 30 ng/mL (22.47% - 20 participants). The minimum vitamin D level detected was 7.4 ng/mL, and the maximum was 54.5 ng/mL (Figure 1).

Our results revealed that men have lower vitamin D levels than women, with a statistically significant difference between gender groups ($p = 0.035$). Compared to men, women may be more aware and educated about the importance of consuming dairy products or supplements to prevent osteoporosis. However, none of the subjects reported the consumption of dietary supplements or medication with vitamin D or calcium in the past three months before the examination.

Table I
Main characteristics of the studied group

Category	Sub-category	Parameter value numerical and (%)
Gender	Men	14 (15.7%)
	Female	75 (84.3%)
Average age		70.09 ± 8.5 years
Age groups	Adults	27 (30.3%)
	Elderly	24 (38.2%)
	Very elderly	23 (25.8%)
	Longevity	5 (5.6%)
Marital status	Unmarried	40 (44.9%)
	Married	49 (55.1%)
Financial status	P-	25 (28.1%)
	P+	64 (71.9%)
Education level	0-6 years	17 (19.1%)
	7-9 years	39 (40.8%)
	> 10 years	33 (37.1%)
Residency	Rural	38 (42.7%)
	Urban	51 (57.3%)
Smoking	Non-smokers	9 (10.1%)
	Smokers	80 (89.9%)

P- below the salary of the middle class in the economy; P+ above the wages of the middle class in the economy

Table II
Blood parameters and cognitive test results

Parameter	Mean \pm SD (min; max)
Vitamin D level (ng/mL)	24.03 ± 10.105 (7.40; 54.50)
Glucose (mg/dL)	115.03 ± 42.44 (59.00; 403.00)
HDL cholesterol (mg/dL)	48.52 ± 11.45 (25.00; 96.00)
LDL cholesterol (mg/dL)	129.38 ± 50.21 (25.11; 400.00)
MOCA	24.39 ± 4.476 (5; 30)
MMSE	27.01 ± 3.186 (14; 30)
CDT	8.93 ± 2.088 (0; 10)
GDS	3.89 ± 3.651 (0; 15)
STRESS	63.67 ± 14.906 (38; 106)
RESILIENCE	67.48 ± 13.400 (39; 100)

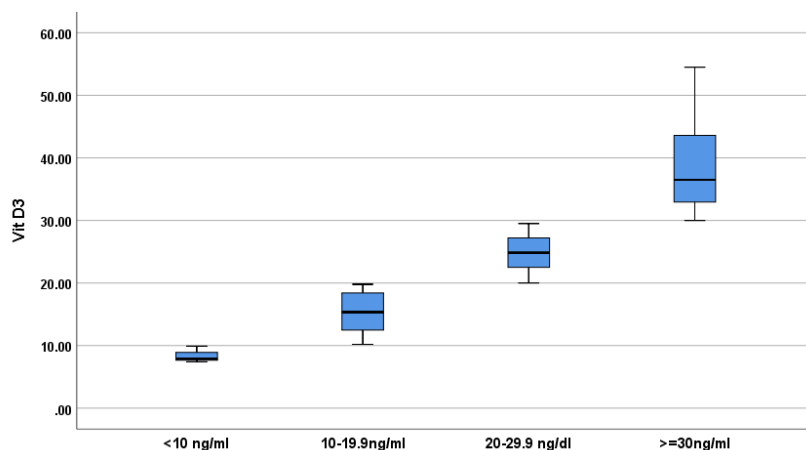


Figure 1.

Vitamin D levels (ng/mL) among study participants: 1) severe vitamin D deficiency: < 10 ng/mL; 2) moderate vitamin D deficiency: between 10 - 19.9 ng/mL; 3) vitamin D relative insufficiency: between 20 - 29.9 ng/mL; 4) adequate level of vitamin D: ≥ 30 ng/mL

By age group, the average level of vitamin D is lower for patients in the 75 - 84 age group (19.66 ng/mL) compared to the younger categories. When comparing participants aged < 65 years with those between 65 - 74 years and 75 - 84 years, statistically significant p values were obtained ($p = 0.016$ and $p = 0.017$, respectively). It can be observed that individuals aged between 75 and 84 years exhibit lower levels of vitamin D compared to those under 65 and those within the age group of 65 to 74 years.

In addition, the one-way ANOVA analysis was performed, with average vitamin D levels for the adult group being 25.45 ± 9.5 ng/mL, similar to the values obtained in the elderly group of 25.81 ± 10.7 ng/mL. In contrast, the very elderly group had an average vitamin D level of 19.66 ± 6.4 ng/mL, the difference being statistically significant ($F = 3.4$, $p = 0.037$). Tukey's post-hoc analysis by age group also revealed a statistically significant difference of 6.1 units (CI 0.13 - 12.1) between the group of elderly and very elderly participants ($p = 0.04$) regarding vitamin D levels in these age groups. Comorbidities, a lack of mobility following the onset of disability, and a lack of active evaluation can explain the decreased levels of vitamin D in the very elderly age group. A recent study calculated an age-related reduction in vitamin D production of 13% *per* decade, demonstrating production at 70 years to be half that at 20 years [13]. Regarding education, 19% of study participants had only 0 - 6 years of education, the lowest percentage,

the rest having a medium or higher level of education (7 - 9 years or more than 10 years of studies). This may be attributed to the fact that those with higher education seek medical help more frequently and notice changes in their functional status more quickly. The average vitamin D level in the group with less than 6 years of education was 21.5 ± 7.9 ng/mL, lower than in the groups with more than 7 years of education. Still, no statistically significant difference was established ($p = 0.55$).

In addition, the average vitamin D level was 21.2 ± 6.7 ng/mL in the smokers' group, lower than that of non-smokers, where the average value was 24.3 ± 9.9 ng/mL, but no statistically significant differences were noted.

According to bivariate Pearson correlation analysis, a weak negative linear relationship ($r = -0.218$, $p = 0.040$) was noted between vitamin D status and the number of associated pathologies. Thus, a low vitamin D level correlates with increased associated diseases (Figure 2). This finding is aligned with other studies, knowing the fact that the elderly population, on the one hand, has limited exposure to the sun, with a low level of vitamin D production, and, on the other hand, presents a large number of comorbidities [12]. Vitamin D also decreases with the onset of stress and influences the occurrence of oncological diseases despite the gene networks [18]. Furthermore, vitamin D status was analysed in relation to cognitive test results. The main findings are shown in Table III.

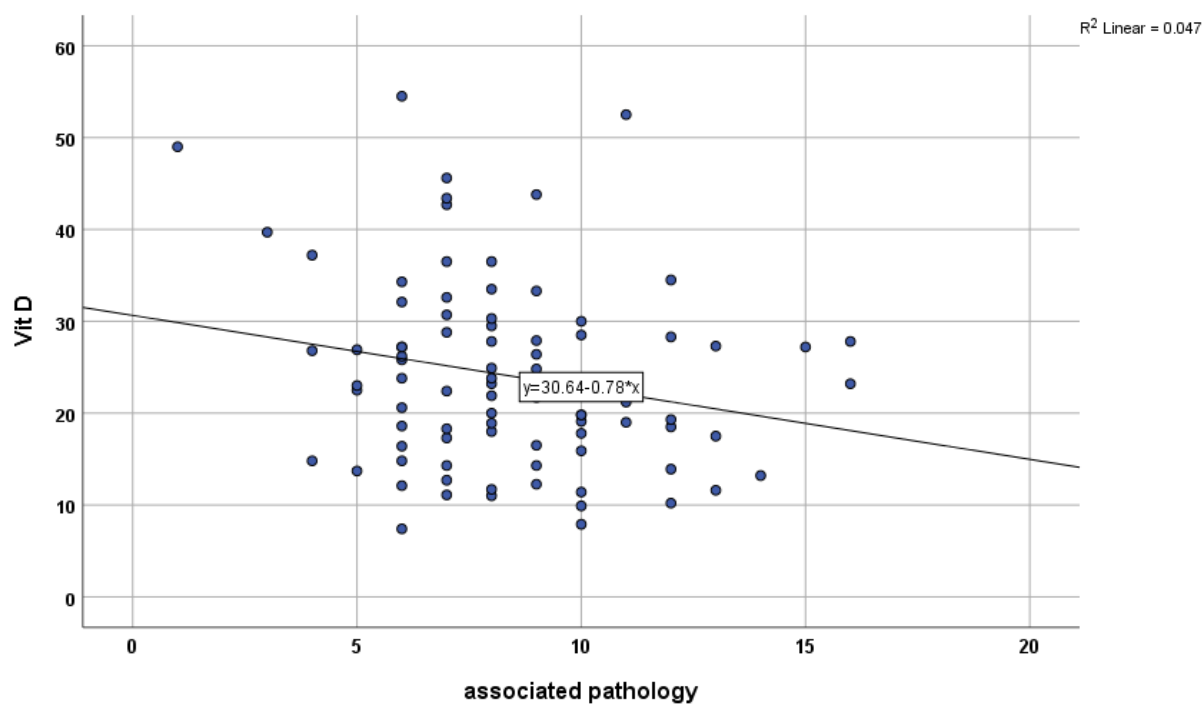


Figure 2.

Vitamin D level (ng/mL) concerning the number of associated diseases in the study group

Table III

Correlations between vitamin D levels and cognitive test results

		MOCA	MMSE	TDC	GDS	STRESS	RESILIENCE
Vitamin D levels	Pearson Correlation	0.256*	0.279**	0.285**	-0.057	0.063	0.005
	Sig. (2-tailed)	0.015	0.008	0.007	0.595	0.559	0.965
MOCA	Pearson Correlation	1	0.793**	0.610**	-0.218*	-0.015	0.114
	Sig. (2-tailed)		0.0001	0.0001	0.040	.0888	0.286
MMSE	Pearson Correlation	0.793**	1	0.595**	-0.192	-0.060	0.223*
	Sig. (2-tailed)	0.0001		0.0001	0.071	0.580	0.035
STRESS	Pearson Correlation	-0.015	-0.060	-0.101	0.616**	1	-0.229*
	Sig. (2-tailed)	0.888	0.580	0.344	0.0001		0.031
RESILIENCE	Pearson Correlation	0.114	0.223*	0.087	-0.231*	-0.229*	1
	Sig. (2-tailed)	0.286	0.035	0.418	0.029	0.031	

* Correlation is significant at the 0.05 level (2-tailed), ** Correlation is significant at the 0.01 level (2-tailed)

Analysing the relationship between vitamin D status and the MMSE test, there is a direct correlation (Pearson correlation coefficient of 0.278, $p < 0.05$); along with vitamin D level decrease, cognitive decline also occurs in studied participants.

In the case of the MOCA test, more minor differences were noted between the percentages of patients who scored greater than or equal to 26 points (55% of all patients) and those who scored less than 26 points (45% of all patients), compared to the results from the MMSE test. Furthermore, after undertaking this test, some participants with adequate vitamin D levels did not achieve a high score of 26 points or more, with 30% scoring less than 26 points. Analysing the correlation between the two variables, a statistically significant weak correlation was found between Vitamin D levels and the score obtained in the MOCA test (Pearson correlation coefficient 0.256, $p < 0.01$).

A percentage of subjects with a MOCA score < 26 points (30%) presented moderate vitamin D deficiency, this relation having a weak statistical correlation but statistically significant (Pearson correlation coefficient 0.256, $p < 0.01$).

Subsequently, an examination was conducted to explore the differences between the two assessments and the criteria for selecting one over the other. The two tests were directly compared with a Pearson correlation coefficient obtained of 0.793, which was statistically significant ($p = 0.024$). We found a statistically significant relationship between the two tests, which differed from previous studies, considering that the studied group does not include many subjects with higher education. It was noted that the average score obtained in the MMSE test was 27.01 points, higher than the MOCA test average of 24.39 points. At the same time, the range of scores in the MMSE test was narrower, ranging between 14 to 30 points, while in the MOCA test, the range was significantly more comprehensive, the minimum score being 5 points and the maximum 30 points.

It can be asserted that although the two assessments have the same objective and characteristics, they can be distinguished by their respective levels of difficulty.

The MOCA test seems to have a higher degree of difficulty, rendering it potentially more effective in identifying early forms of dementia, thus having a higher sensitivity. Nonetheless, it should be noted that this heightened sensitivity may also lead to an increase in the proportion of false positive results, resulting in decreased specificity. However, it could be challenging to apply in the case of patients with advanced forms of cognitive impairment due to this increased degree of difficulty. Applying this assessment tool to patients with advanced cognitive impairment can pose significant challenges due to the complexity involved. In cases of advanced dementia, utilising a test with heightened sensitivity may not be advisable, as it risks inducing frustration among individuals who are unable to complete the test successfully. The MMSE appears to be more appropriate for moderate and severe forms of cognitive impairment, being a test with a lower degree of difficulty but which may not detect mild forms of dementia.

The CDT, a useful tool for assessing cognitive decline, is relatively straightforward in its application. The correlation between the CDT and resilience test ($r = 0.223$, $p = 0.035$) is important in evaluating participants' intrinsic capacity and stress response, allowing for their subsequent active monitoring. The CDT is a screening assessment for mild cognitive impairment recommended by many professional societies, such as cardiology and/or diabetes [24]. Table III shows a moderate positive correlation between vitamin D levels and CDT score ($r = 0.331$, $p = 0.002$).

Several studies found that low levels of vitamin D are associated with more severe cases of cognitive impairment, especially in older individuals or those diagnosed with Alzheimer's disease [32, 36]. In these situations, supplementing vitamin D could slow the progression of cerebral degeneration [41]. This measure is important because previous studies have found low vitamin D levels in the elderly Romanian population, especially in senior women [8, 27, 41]. Recent studies noted a prevalence of vitamin D deficiency in the adult population of 24.8% [10, 33].

Further research revealed that higher vitamin D levels increase cognition, especially executive function and mental flexibility [21, 52]. Several possible biological mechanisms are described in the literature by which vitamin D might influence cognitive function. Vitamin D's antioxidant effects may protect cultured human endothelial cells and retinal cone cells from oxidative stress [35]. Furthermore, vitamin D is implicated in neuroprotection and neuromodulation pathways binding to vitamin D receptors, calcium homeostasis, modulation of oxidative stress and inhibition of inflammatory processes in the brain [5, 28]. Early childhood vitamin D insufficiency has been associated with elevated arterial blood pressure, enhanced vascular oxidative stress and alterations in cardiac gene expression [43].

Nevertheless, the degree of stress and resilience of the participants in this study was not influenced by their vitamin D levels (Pearson correlation coefficient of 0.062). Applying the ANOVA analysis for the MOCA test resulted in an average level of 26.25 ± 9.9 ng/mL of vitamin D for the group without depression. In contrast, the group with depression presented an average vitamin D level of 21.3 ± 9.7 ng/mL, a lower value with a statistically significant difference ($F = 5.5$, $p = 0.021$).

Other research has shown that vitamin D status is associated with trait resilience but not depression in a general population [47].

At the same time, recent studies demonstrated that subjects exposed to an experimental stress procedure have higher vitamin D concentrations; thus, increased vitamin D levels are related to higher resilience [23]. Moreover, stress and stress resilience depend on a number of factors, more prevalent in older adult populations, such as various pathologies, difficult financial situations and the absence of social support. These factors often lead to the resumption of work, which accentuates their stress [39].

Research regarding the association between cognitive impairment and vitamin D status frequently yields inconsistent results, necessitating additional studies to elucidate this relationship. Research indicates that older adults with adequate vitamin D levels exhibit a reduced prevalence of cognitive impairment [34].

Significant associations were identified between vitamin D levels and the Mini-Mental State Examination, independent of cognitive impairment status. Vitamin D has been shown to have various beneficial effects on neural and endothelial dysfunctions, which may elucidate its protective role in neurodegenerative processes [6, 40]. Furthermore, vitamin D supplementation effectively decreased amyloid β -related biomarkers in individuals diagnosed with Alzheimer's [26].

On the other hand, factors contributing to the risk of vitamin D deficiency in the elderly include relative resistance to vitamin D's role in increasing calcium absorption in the gastrointestinal tract and a decline

in renal function associated with ageing. The ageing kidney exhibits a diminished capacity to convert 25-hydroxyvitamin D (25(OH)D) into 1,25-dihydroxyvitamin D. In addition to sun exposure, skin phenotype and dietary intake, other variables affect vitamin D levels in the elderly population. Smoking may reduce serum 25(OH)D concentrations, although the mechanism remains unidentified [14]. An increased proportion of total body fat also correlates with diminished 25(OH)D circulating levels. Fat content remains a variable despite adjustments for age, season and smoking in both men and women. This seems to involve diminished production and increased distribution volume of vitamin D [45]. The global rise in BMI among the ageing population underscores obesity as a significant contributor to reduced levels of 25(OH)D [37].

Vitamin D supplementation can be done pharmacologically or through food fortification for diagnosed deficiency. Treatment goals should focus on avoiding 25(OH)D serum levels < 12 ng/mL to reach levels > 20 ng/mL [16].

Elderly patients consume many drugs, mainly due to polypharmacy. That is why it is preferable to administer a mixed supplement of vitamin D together with vitamin C, both of which increase resilience and reduce inflammation [13, 33].

Limitations of the study

The limited number of patients enrolled in this study can be attributed to the restricted timeframe available for research and inadequate funding, which were the most significant factors influencing the sample size. As previously noted, our findings reveal a lack of correlations between vitamin D status, depression, stress and resilience, likely due to the small sample size. Consequently, further research is required to elucidate these relationships.

Conclusions

Our findings revealed that the prevalence of vitamin D deficiency increases with age. According to our research, vitamin D may help prevent cognitive decline primarily by enhancing memory function following the onset of age-related changes and neurodegenerative diseases. Higher levels of stress and resilience modification were linked to vitamin D insufficiency in older persons. This offers a solid scientific foundation for evaluating the impact of vitamin D supplementation on behavioural issues in elderly individuals who are vitamin D deficient. Mounting data suggest that vitamin D and C may help improve older adults' immune system performance, cardiovascular health and cognitive function. These vitamins are anticipated to ultimately be incorporated into a single formulation, thereby reducing the necessity for multiple medications, which represent another stress factor in the elderly population.

Conflict of interest

The authors declare no conflict of interest.

References

1. Aprahamian I, Martinelli JE, Neri AL, Yassuda MS, The Clock Drawing Test: A review of its accuracy in screening for dementia. *Dement Neuropsychol.*, 2009; 3(2): 74-81.
2. Aunan JR, Watson MM, Hagland HR, Søreide K. Molecular and biological hallmarks of ageing. *Br J Surg.*, 2016; 103(2): e29-e46.
3. Aurelian SM, Ciobanu A, Cărare R, Stoica SI, Angheliescu A, Ciobanu V, Onose G, Munteanu C, Popescu C, Andone I, Spînu A, Firan C, Cazacu IS, Trandafir AI, Băilă M, Postoiu RL, Zamfirescu A, Topical Cellular/Tissue and Molecular Aspects Regarding Nonpharmacological Interventions in Alzheimer's Disease-A Systematic Review. *Int J Mol Sci.*, 2023; 24(16533): 1-29.
4. Aurelian SM, Stanciu OM, Zamfirescu A, Aurelian J, Nica AS, Capisizu A, A possible link between frailty and diabetes. 2nd International Conference on Interdisciplinary Management of Diabetes Mellitus and its Complications, *Interdiab 2016: Diabetes Mellitus As Cardiovascular Disease*, pp.79-88.
5. Bivona G, Gambino CM, Iacolino G, Ciaccio M, Vitamin D and the nervous system. *Neurol Res.*, 2019; 41(9): 827-835.
6. Bivona G, Lo Sasso B, Gambino CM, Giglio RV, Scazzone C, Agnello L, Ciaccio M, The role of vitamin D as a biomarker in Alzheimer's disease. *Brain Sci.*, 2021; 11(3): 334-340.
7. Bădileanu M, Ionel IP, Aurelian J, Cristian DA, Jude C, Georgescu LI, Răpan I, Perception and Deception in Nurses' Clinical and Work-Related Professional Autonomy: Case Study for a Hospital in Romania. *Sustainability*, 2023; 15(355): 1-19.
8. Bischoff-Ferrari HA, Dawson-Hughes B, Willett WC, Staehelin HB, Bazemore MG, Zee RY, Wong JB, Effect of Vitamin D on falls: a meta-analysis. *JAMA*, 2004; 291(16): 1999-2006.
9. Blanke ES, Schmiedek F, Siebert S, Richter D, Brose A, Perspectives on resilience: Trait resilience, correlates of resilience in daily life, and longer-term change in affective distress. *Stress Health*, 2023; 39(1): 59-73.
10. Brîndușe LA, Eclemea I, Neculau AE, Cucu MA, Vitamin D Status in the Adult Population of Romania-Results of the European Health Examination Survey. *Nutrients*, 2024; 16(6): 867.
11. Bucurica S, Nancoff AS, Dutu M, Mititelu MR, Gaman LE, Ioniță-Radu F, Jinga M, Maniu I, Ruța F, Exploring the Relationship between Lipid Profile, Inflammatory State and 25-OH Vitamin D Serum Levels in Hospitalized Patients. *Biomedicines*, 2024; 12(8): 1686.
12. Capisizu A, Zamfirescu A, Aurelian SM, Dina I, Results of prevalence study regarding comorbidities for various patients. *Rom Stat Rev.*, 2013; 5: 75-87.
13. Chalcraft, JR, Cardinal LM, Wechsler PJ, Hollis BW, Gerow KG, Alexander BM, Keith JF, Larson-Meyer DE, Vitamin D Synthesis Following a Single Bout of Sun Exposure in Older and Younger Men and Women. *Nutrients*, 2020; 12(8): 2237-2251.
14. Cutillas-Marco E, Fuertes-Prosper A, Grant WB, Morales-Suarez-Varela M, Vitamin D deficiency in South Europe: effect of smoking and aging. *Photodermatol Photoimmunol Photomed.*, 2012; 28(3): 159-161.
15. Cherniack EP, Troen BR, Florez HJ, Roos BA, Levis S, Some new food for thought: the role of vitamin D in the mental health of older adults. *Curr Psychiatry Rep.*, 2009; 11(1): 12-19.
16. Dautzenberg G, Lijmer J, Beekman A, Diagnostic accuracy of the Montreal Cognitive Assessment (MoCA) for cognitive screening in old age psychiatry: Determining cutoff scores in clinical practice. Avoiding spectrum bias caused by healthy controls. *Int J Geriatr Psychiatry*, 2020; 35(3): 261-269.
17. Dragan GR, Filip C, Badulescu OV, Badescu MC, Filip C, Bild W, Dobrin I, Dragan AG, Serban R, Diaconu IE, Ciobica A, Ciocoiu M, Vitamin C treatment improves recalling scores in Mini-Mental State Examination in patients with cognitive impairment. *Farmacia*, 2023; 71(6): 1165-1173.
18. Filippi A, Aurelian J, Mocanu M-M, Analysis of the Gene Networks and Pathways Correlated with Tissue Differentiation in Prostate Cancer. *Int J Mol Sci.*, 2024; 25(7): 3626.
19. Gao Q, Kou T, Zhuang B, Ren Y, Dong X, Wang Q, The Association between Vitamin D Deficiency and Sleep Disorders: A Systematic Review and Meta-Analysis. *Nutrients*, 2018; 10(10): 1395.
20. Giustina A, Bouillon R, Dawson-Hughes B, Ebeling PR, Lazaretti-Castro M, Lips P, Marcocci C, Bilezikian JP, Vitamin D in the older population: a consensus statement. *Endocrine*, 2023; 79(1): 31-44.
21. Goodwill AM, Campbell S, Simpson S Jr, Bisignano M, Chiang C, Dennerstein L, Szoek C, Vitamin D status is associated with executive function a decade later: Data from the Women's Healthy Ageing Project. *Maturitas*, 2018; 107: 56-62.
22. Grant WB, Ecological studies of the UVB-vitamin D-cancer hypothesis. *Anticancer Res.*, 2012; 32(1): 223-236.
23. Hansen AL, Ambroziak G, Thornton D, Mundt JC, Kahn RE, Dahl L, Waage L, Kattenbraker D, Araujo P, Murison R, Rypdal K, Grung B, Vitamin D Supplementation during Winter: Effects on Stress Resilience in a Randomized Control Trial. *Nutrients*, 2020; 12(11): 3258.
24. Higaki Y, Clock-drawing Test and Cube-copying Test to Quickly Screen Dementia: In Combination with the Mini-mental State Examination Scores. *Intern Med.*, 2024; 63(9): 1223-1228.
25. Hossain S, Beydoun MA, Beydoun HA, Chen X, Zonderman AB, Wood RJ, Vitamin D and breast cancer: A systematic review and meta-analysis of observational studies. *Clin Nutr ESPEN*, 2019; 30: 170-184.
26. Jia J, Hu J, Huo X, Miao R, Zhang Y, Ma F, Effects of vitamin D supplementation on cognitive function and blood A β -related biomarkers in older adults with Alzheimer's disease: a randomised, double-blind, placebo-controlled trial. *J Neurol Neurosurg Psychiatry*, 2019; 90(12): 1347-1352.

27. Kiraly S, Kiraly M, Hawe R, Vitamin D as a neuroactive substance: review. *Sci World J.*, 2006; 26: 125-139.
28. Lason W, Jantas D, Leškiewicz M, Regulska M, Basta-Kaim A, The Vitamin D Receptor as a Potential Target for the Treatment of Age-Related Neurodegenerative Diseases Such as Alzheimer's and Parkinson's Diseases: A Narrative Review. *Cells*, 2023; 12(4): 660.
29. Levenstein S, Prantera C, Varvo V, Scribano ML, Berto E, Luzi C, Andreoli A, Development of the Perceived Stress Questionnaire: a new tool for psychosomatic research. *J Psychosom Res.*, 1993; 37(1): 19-32.
30. Li H, Sun D, Wang A, Pan H, Feng W, Ng CH, Ungvari GS, Tao L, Li X, Wang W, Xiang YT, Guo X, Serum 25-Hydroxyvitamin D Levels and Depression in Older Adults: A Dose-Response Meta-Analysis of Prospective Cohort Studies. *Am J Geriatr Psychiatry*, 2019; 27(11): 1192-1202.
31. Li S, Xi C, Li L, Long Z, Zhang N, Yin H, Xie K, Wu Z, Tian J, Wang F, Wang M, Comparisons of different vitamin D supplementation for prevention of osteoporotic fractures: a Bayesian network meta-analysis and meta-regression of randomised controlled trials. *Int J Food Sci Nutr.*, 2021; 72(4): 518-528.
32. Lu Y, Li J, Hu T, Huang G, Serum 25-hydroxy vitamin D level is associated with cognitive impairment in people aged 65 years and older. *Ann Palliat Med.*, 2021; 10(7): 7479-7485.
33. Marti DT, Nesi A, Balta C, Olariu TR, Mihiu AG, Hermenean, A, Oatis DA, Retrospective Analysis of Vitamin D Deficiency in an Adult Population of Arad County, Western Romania (2019-2022). *Life*, 2024; 14(2): 274.
34. Matsuo LH, Confortin SC, Ceolin G, Soar C, Xavier AJ, D'Orsi E, Moreira JD, Association between lower serum vitamin D (25-hydroxy-cholecalciferol) concentrations and cognitive impairment in older adults: data from a populational-based cohort study in a middle-income country. *Public Health Nutr.*, 2022; 25(9): 2507-2516.
35. Miao D, Goltzman D, Mechanisms of action of vitamin D in delaying aging and preventing disease by inhibiting oxidative stress. *Vitam Horm.*, 2023; 121: 293-318.
36. Murdaca G, Banchemo S, Tonacci A, Nencioni A, Monacelli F, Gangemi S, Vitamin D and Folate as Predictors of MMSE in Alzheimer's Disease: A Machine Learning Analysis. *Diagnostics*, 2021; 11(6): 940.
37. NCD Risk Factor Collaboration (NCD-RisC), Rising rural body-mass index is the main driver of the global obesity epidemic in adults. *Nature*, 2019; 569(7755): 260-264.
38. Nicolescu LC, Popescu CL, Popescu CV, Nicolescu CM, Nesi A, Pilat L, Stanciu AN, Mihiu AG, The evaluation of vitamin D deficiency as a risk factor in the case of patients with moderate COVID-19. *Farmacia*, 2022; 70(3): 507-513.
39. Oancea C, Cernamoriti A, Gherman DM, Popescu FG, Social Insurance Physician Burnout-Stress Factors and Coping Strategies. *Medicina (Kaunas)*, 2023; 59(3): 436.
40. Perez L, Heim L, Sherzai A, Jaceldo-Siegl K, Nutrition and vascular dementia. *J Nutr Health Aging.*, 2012; 16(4): 319-324.
41. Pinzon RT, Christina NS, Sugianto S, Primastuti T, Correlation between vitamin D levels and cognitive impairment on 90 days post ischemic stroke patients. *Ro J Neur.*, 2022; 21(3): 237-241.
42. Pocinho MTS, Farate C, Dias CA, Lee TT, Yesavage JA, Clinical and Psychometric Validation of the Geriatric Depression Scale (GDS) for Portuguese Elders. *Clinical Gerontologist.*, 2009; 32(2): 223-236.
43. Rihal V, Khan H, Kaur A, Singh TG, Vitamin D as therapeutic modulator in cerebrovascular diseases: a mechanistic perspectives. *Crit Rev Food Sci Nutr.*, 2023; 63(25): 7772-7794.
44. Sharma Y, Popescu A, Horwood C, Hakendorf P, Thompson C, Relationship between Vitamin C Deficiency and Cognitive Impairment in Older Hospitalised Patients: A Cross-Sectional Study. *Antioxidants*, 2022; 11(3): 463.
45. Snijder MB, van Dam RM, Visser M, Deeg DJ, Dekker JM, Bouter LM, Seidell JC, Lips P, Adiposity in relation to vitamin D status and parathyroid hormone levels: a population-based study in older men and women. *J Clin Endocrinol Metab.*, 2005; 90(7): 4119-4123.
46. Sultan S, Taimuri U, Basnan SA, Ai-Orabi WK, Awadallah A, Almowald F, Hazazi A, Low Vitamin D and Its Association with Cognitive Impairment and Dementia. *J Aging Res.*, 2020; 2020: 6097820.
47. Terock J, Hannemann A, Janowitz D, Müller J, Völzke H, Grabe HJ, Vitamin D levels are associated with trait resilience but not depression in a general population sample. *Brain Behav.*, 2020; 10(12): e01884.
48. Trandafir AI, Onose G, Munteanu C, Băila M, Saqlam AO, Mandu M, Săulescu I, Grădinaru E, Bojincă VC, Particularities regarding Clinical-biological and Evolutionary Parameters of Immune-mediated Rheumatic Diseases in Patients with COVID-19 – systematic literature review. *Balneo PRM Res J.*, 2023; 14(2): 562-585.
49. Valer-Martinez A, Martinez JA, Sayon-Orea C, Galvano F, Grosso G, Bes-Rastrollo M, Vitamin D and Cardio-Metabolic Risk Factors in Overweight Adults: An Overview of the Evidence. *Curr Pharm Des.*, 2019; 25(22): 2407-2420.
50. Watfa G, Husson N, Buatois S, Laurain MC, Miget P, Benetos A, Study of Mini-Mental State Exam evolution in community-dwelling subjects aged over 60 years without dementia. *J Nutr Health Aging.*, 2011; 15(10): 901-904.
51. World Health Organisation (WHO). Stress: Questions and answers. 2023, www.who.int/news-room/questions-and-answers/item/stress.
52. Zucic Soares J, Pettersen R, Saltyte Benth J, Knapskog AB, Selbæk G, Bogdanovic N, Higher Vitamin D levels are associated with better attentional functions: Data from the NorCog Register. *J Nutr Health Aging.*, 2019; 23(8): 725-731.
53. Zugravu CA, Soptica F, Tarcea M, Cucu A, Pertinence of Vitamin D supplementation in the adult Romanian population. *Farmacia*, 2016; 64(3): 467-472.