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25-hydroxyvitamin D status and its impact on cognitive functions in postmenopausal woman

Poziom 25-hydroksywitaminy D i jego wpływ na funkcje kognitywne u kobiet po menopauzie

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Summary

Aim: The purpose of the study was to analyze the cognitive functions in postmenopausal women carriers of Apolipoprotein E gene polymorphisms (APOE) with different status of vitamin D levels.

Material/Methods: 170 ambulatory individuals aged 50 years or older were evaluated. A computerized battery of Central Nervous System Vital Signs (CNS VS) was used for diagnostic cognitive functions. APOE genotype was performed by multiplex PCR. Serum 25(OH)D and estradiol levels were measured using the 25OHD EIA assay and Estradiol ELISA Kits.

Results: Considerably worse scores in global cognitive performance index (NCI) were obtained by women with severe deficiency of 25(OH)D ($p < 0.001$). The cognitive effects of very low 25(OH)D levels were apparent in memory, executive functioning, complex attention, and cognitive flexibility. The genotype APOE ε3/ε4 or ε4/ε4 were most common (19.6%; 15.2%, respectively) in women with 25(OH)D severe deficiency which had the weakest average results in terms of NCI value.

Conclusions: The severe deficiency of 25(OH)D vitamin was related with a greater likelihood of cognitive impairment and risk of cognitive decline in postmenopausal women with no dementia.

Keywords: 25-hydroxyvitamin D • Cognitive function • the battery of computer tests-CNS-VS • Postmenopausal women

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INTRODUCTION

The clinical study showed that low serum concentrations of 25-hydroxyvitamin D [25(OH)D] have been associated with increased risk of mortality, cardiovascular disease (CVD), and diabetes [16, 20, 21]. Recent meta-analyses also confirm that low serum vitamin D concentrations are associated with an increased risk of cognitive decline and dementia particularly among the elderly populations [23]. Other studies reported that there exists an association between vitamin D levels and cognitive impairment in women, but not in men [24]. However, results of studies published on this topic are inconsistent, i.e., not reporting a significant association of 25(OH)D with cognition and dementia [18, 19, 23]. Currently, the best available indicator of vitamin D status is circulating serum 25(OH)D, which is taken up by target cells and would determine the risk of age-related chronic diseases. Adequate plasma 25(OH)D levels seem to be between 30 ng/mL and 90 ng/mL, though there is no international consensus [11]. Hypovitaminosis D includes three categories according to cutoff values: insufficiency if serum 25(OH)D levels are between 20 and 30 ng/mL, deficiency below 20 ng/mL, and severe deficiency below 10 ng/mL but again there is controversy about absolute levels [21].

The cognitive effects of estradiol are mediated at sites and/or neural systems in the cerebral cortex, basal forebrain, hippocampus and striatum that regulate higher order neural function [14]. Therefore, in postmenopausal women, the effect of the reduced sex hormones, especially estrogen, as well as other factors including homocysteine, lipids, polymorphism of APOE gene affecting cognitive disorders are of particular interest [4, 22].

We aimed to test the hypothesis that very low 25(OH)D levels are associated with a greater likelihood of cognitive impairment and increased risk of cognitive decline in postmenopausal women. To achieve the aim, we assessed cognitive function using the computerized neurocognitive tests known as Central Nervous System Vital Signs (CNS-VS) in postmenopausal women, carriers of Apolipoprotein E gene polymorphisms, having different status of vitamin D levels.

METHODS

Study design and participants

The study was conducted in 2013 at the Institute of Rural Health in Lublin, Poland, and the study group consisted of women from the south-eastern part of the country. A total of 170 postmenopausal women were recruited. The subjects were screened using a questionnaire, with the following inclusion criteria: healthy postmenopausal women aged 50 to 65 years, with absence of menstrual cycles for at least two years and at least completed primary school.

The exclusion criteria were screened by questionnaire and structured interview with patients. Subjects who had active cancer disease within the period of 5 years from recruiting, mental diseases in the interview including depression in the premenopausal period, pharmacological and alcohol additions and/or diagnosed disease entity with dementia symptoms, were excluded from the study.

The women were also qualified into that examined group on the grounds of clinical symptoms (minimum 2 years from the last menstruation) as well as on the basis of the follicle-stimulating hormone (FSH) concentration (FSH >30 mlU/ml).

At the qualification stage, short test MoCA (Montreal Scale of Cognitive Function Assessment) was conducted in order to include the female patients who did not show dementia signs. MoCA test was designed as a fast screening tool used for the assessment of mild cognitive dysfunction. The maximum number of points in this test amounts to 30, and the score of 26 points or more is considered to be correct. All women examined who were qualified for further stages of the study obtained more than 26 points in MoCA test.

Postmenopausal women were grouped according to 25(OH)D concentration ~~25(OH)D~~ vitamin deficiency [23] as follows: severe deficiency below 10 ng/mL (group I); deficiency below 20 ng/mL (group II); insufficiency between 20 and 30 ng/mL (group III), and normal levels of 25(OH)D >30 ng/mL (group IV), and their cognitive functions were analyzed by CNS-VS in accordance with groups of 25(OH)D.

COGNITIVE FUNCTION ASSESSMENT

The assessment of cognitive function was made with the use of diagnostic equipment CNS-Vital Signs (Polish version), on the basis of software from the CNS Vital Signs Company, on 1829 East Franklin Street, Bldg 500, Chapel Hill NC 27514, 919-933-0932. The test battery consisted of the following: The Verbal Memory Test (VBM), Test of Motor Functioning – Finger Tapping Test (FTT), Symbol Digit Modalities Test (SDMT), Stroop Test (ST), Shifting Attention Test (SAT) and The Continuous Performance Test. Neurocognitive Index (NCI) was calculated on the basis of 5 domains: memory, psychomotor speed, reaction time, attention and cognitive plasticity. The following cognitive functions were evaluated as domains: memory, verbal memory, visual memory, speed of processing, executive functions, psychomotor speed, reaction time, attention focusing and cognitive plasticity.

The average standardized results were used for calculations, which enabled comparisons. CNS Vital Signs, on the basis of standard scores, qualifies the obtained results of a particular cognitive function into five categories: above (more than 110 standard score points), average (90–109), below average (80–89), poor (70–79) and very poor (less than 70) [10].

GENETIC STUDY AND BIOCHEMICAL ANALYSIS

Genomic DNA isolation was extracted from 0.2 ml of human whole blood by QIAamp DNA Blood Mini Kit (Qiagen, USA) according to the producer's instructions. In the study, genotyping methods were applied based on the detection of variations in the nucleotide sequences of alleles of APOE genes (single nucleotide polymorphism, SNP). Multiplex PCR was done according to Yang et al. [26] with some modifications.

Fasting morning blood was collected; serum was prepared immediately after phlebotomy and then stored at – 81°C. Serum levels of 25(OH)D and estradiol were measured using the 25OHD EIA assay and Estradiol ELISA Kits (R&D Systems Minneapolis, USA). Lipid profile was determined using an automatic biochemistry analyzer Express Plus (Chiron Diagnostics, USA), with reagents by Siemens (Siemens Healthcare Diagnostics, Tarrytown, NY, USA), according to the procedure provided by the manufacturer. FSH was detected with electrochemiluminescence immunoassay using an automatic device (ARCHITECT Plus i2000SR, Abbott Diagnostics, IL, USA).

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

STATISTICAL ANALYSIS

For the parameters of the characteristics of the study population (Tab. 1), the results were presented as arithmetic means (Mean) ± standard deviation (SD) and for quantitative characteristics and/or absolute (n) and relative numbers (%) for qualitative characteristics. The significance of the differences in quantitative characteristics between four groups of 25-hydroxyvitamin D was investigated using analysis of variance F test. The normal distribution of these variables was tested by the Shapiro-Wilk test.

Analysis of cognitive functions covered the minimum–maximum levels (Min-max), mean ± standard deviation (Mean (SD), and median (Me). The significance of the differences in cognitive functions between groups of was examined using H Kruskal-Wallis test. Spearman's correlation coefficient was used for correlation analyses. The level of significance – $p < 0.05$ was accepted. Statistical analyses were performed with GraphPad Prism software.

The present study was approved by the Ethics Committee of the Institute of Rural Health in Lublin, Poland.

RESULTS

The characteristics of study population by 25(OH) status are detailed in Table 1.

The studied women were matched for age, education, environment, BMI, and they had a similar reproductive period. The mean age of all participants was 56.45 ± 3.54 years. BMI averaged 26.44 ± 4.14 kg/m^2, and participants had an average reproductive period of 37.28 ± 3.02 years. This was a highly educated group with an average of 13.63 ± 2.86 years of education, which mainly lived in towns (84.7%).

The levels of estradiol and lipids: total cholesterol, HDL cholesterol, LDL cholesterol, and trigliceride were: 22.3 ± 2.9 pg/ml and 11.5 22.4 ± 20.0 mg/ml; 228.3 ± 44.0 mg/ml; 54.4 ± 12.5 mg/ml; 147.6 ± 63.6 mg/ml, respectively.

The mean 25(OH)D serum concentrations were 21.48 ± 12.9 ng/ml and it was correlated with estradiol levels ($p = 0.042$). Moreover, there were no significant differences in age, years of education, body mass index, reproductive period, estradiol and lipids levels between the 25-hydroxyvitamin D groups, using analysis of variance F test (Tab. 1). Interestingly, only 36.9% of women with severe vitamin D deficiency used hormone replacement therapy (HRT), while in other groups this percentage was 54.8% – 66.8% (Tab. 1).

The frequency of different polymorphism of apo E gene in studied patients is shown in Table 1. Genotype APOE ε4/ε4 occurred in 10 examined women, which represented 5.9% of the sample while ε3/ε4 polymorphism was found in 25 (14.7%), ε3/ε3 in 104 (61.2%) and ε2/ε3 in 31(18.2%) of women.

The percentage of the genotype APOE ε3/ε4 or ε4/ε4 in women with 25(OH)D severe deficiency (>10 ng/mL) was 19.6% and 15.2%, while in the groups of patients with deficiency 16.3% and 0.0%; with insufficiency 11.7% and 4.6%, and normal levels of 25(OH)D 11.4% and 2.3%, respectively.

The analysis of the changes in the cognitive functions is shown in Table 2.

We revealed that NCI value differed considerably among four 25(OH)D groups ($p = 0.0005$). The considerably better NCI was obtained by women with normal levels of 25(OH)D (<30 ng/ml) in comparison with those with severe deficiency 25(OH)D (>10 ng/ml) ($p < 0.001$); and in both with deficiency 25(OH)D (10–20 ng/ml) and insufficiency 25(OH)D (20–30 ng/ml), ($p < 0.05$).

Similarly, memory and cognitive flexibility differed considerably among 25(OH)D groups ($p = 0.006$; $p = 0.0004$, respectively) and both, verbal memory ($p = 0.0037$) and complex attention ($p = 0.0276$). Moreover, other cognitive functions, i.e., visual memory ($p = 0.2297$), processing speed ($p = 0.2952$), executive functioning ($p = 0.3306$), psychomotor speed ($p = 0.0527$), and reaction time ($p = 0.0819$), did not differ considerably among the 25(OH)D groups.

Women with normal 25(OH)D levels had much better cognitive flexibility ($p < 0.001$) and slightly better memory and verbal memory ($p < 0.01$), psychomotor speed, complex attention ($p < 0.05$) than women with severe deficiency 25(OH)D.

Table 1. Characteristics of the study population

	Prevalence, % 25-hydroxyvitamin D			
Characteristics	**<10 ng/mL**	**10–20 ng/mL**	**20–30 ng/mL**	**>30 ng/mL**
	Gr I, n = 46	**Gr II, n = 37**	**Gr III, n = 43**	**Gr IV, n = 44**
Age, years; Mean (SD)*	56.4 (3.4)	56.5 (3.6)	57.0 (3.8)	55.9 (3.4)
Education, years; Mean (SD)*	13.2 (3.0)	13.4 (2.9)	13.8 (2.7)	14.1 (2.9)
8 years	4.3	5.4	0.0	4.5
9-12 years	60.9	59.6	60.5	47.7
>12 years	34.8	35.0	39.5	47.7
Body Mass Index, kg/m2; Mean (SD)*	26.8 (4.5)	27.2 (4.1)	25.8 (4.2)	26.3 (3.5)
<18.5	0.0	2.7	4.6	0.0
18.5-30	76.1	70.3	79.1	86.4
>30	23.9	27.0	16.3	13.6
Environment: Town	47.8	62.2	67.4	56.8
Little town	36.9	29.7	16.3	22.7
Village	15.3	8.1	16.3	20.5
Reproductive period, years; Mean (SD)*	37.6 (2.8)	37.4 (2.5)	37.0 (3.2)	37.1 (3.4)
25-hydroxyvitamin D, ng/mL Mean (SD)	8.2 (1.1)	14.0 (2.8)	25.2 (3.0)	38.4 (8.1)
Estradiol, pg/ml, Mean (SD)*	20.5 (17.2)	19.1 (18.9)	24.2 (18.4)	25.4 (24.7)
<10	39.1	40.5	20.9	40.9
10-30	41.3	48.6	55.8	31.8
>30	19.6	10.9	23.3	27.3
Blood pressure (mmHg)				
<140/90	78.3	56.7	62.8	77.3
>140/90	21.7	43.3	37.2	22.7
Hormone replacement therapy	36.9	54.8	55.8	66.8
APOE gene polymorphisms:				
ε2/ε3	13.0	10.8	27.9	20.4
ε3/ε3	52.2	72.9	55.8	65.9
ε3/ε4	19.6	16.3	11.7	11.4
ε4/ε4	15.2	0.0	4.6	2.3
Lipids: (mg/ml); Mean (SD)				
Total Cholesterol*	234.7 (46.4)	230.0 (44.7)	229.8 (36.6)	218.8 (47.3)
Triglycerides*	144.4 (66.7)	158.2 (69.0)	142.8 (55.2)	146.6 (64.5)
LDL-cholesterol*	155.5 (46.0)	140.9 (50.6)	142.8 (55.2)	137.7 (46.5)
HDL-cholesterol*	50.3 (12.8)	57.4 (12.7)	58.7 (12.2)	51.8 (10.4)

*There were no significant differences in age, years of education, body mass index, reproductive period, estradiol and lipids levels between the 25-hydroxyvitamin D groups, using analysis of variance F test.

In addition, much better results in the field of cognitive flexibility ($p < 0.01$), memory ($p < 0.01$) and verbal memory ($p < 0.05$) exhibited women with insufficiency 25(OH)D levels in comparison with those with severe deficiency 25(OH)D.

In the present work, we used the clinical levels of severity (Tab. 3), which contains five degrees based on a database with more than 1.900 subjects [15].

The mean NCL of participants was 81.19 ± 17.8 pts., which corresponds with the low average level. The average results in terms of memory, executive functions, psychomotor speed, reaction time, attention focusing, and cognitive plasticity were 89.66 ± 14.8; 80.10 ± 24.5; 82.63 ± 17; 86.61 ± 16.3; 83.58 ± 26.7 and 86.5 ± 20.1 pts., respectively, which correspond with the low average level (Tab. 3).

Table 2. Analysis of cognitive functions in particular groups with 25-hydroxyvitamin D levels

		Domain								
Vitamin	**NCI**	**Memory**	**Verbal Memory**	**Visual memory**	**Processing speed**	**Executive functioning**	**Psychomotor speed**	**Reaction time**	**Complex attention**	**Cognitive flexibility**
25(OH)D >10 ng/mL	**n = 46**									
Min – max	29–100	56–104	51–106	47–119	26–101	21–114	29–109	47–121	16–118	18–108
Mean (SD)	71.52 (18.1)	81.91 (11.8)	82.17 (14.4)	90.22 (16.6)	76.47 (13.5)	75.28 (25.1)	76.74 (18.4)	87.50 (17.7)	74.26 (29.9)	74.78 (23.7)
Me	71	84.5	79.5	90	76	83.5	76.5	90.5	86	82.5
25(OH)D 10–20 ng/mL	**n = 37**									
Min – max	48–108	65–118	41–125	62–122	40–111	25–117	22–109	50–104	16–118	51–115
Mean (SD)	84.19 (15.5)	94.24 (13.2)	87.05 (20.1)	96.24 (13.4)	76.35 (13.9)	83.00 (23.8)	82.57 (18.1)	81.41 (14.0)	86.30 (26.1)	89.22 (17.0)
Me	86	95	93	100	81	88	87	84	96	92
25(OH)D 20–30 ng/mL	**n = 43**									
Min – max	44–109	61–120	49–118	59–119	30–106	33–111	42–111	36–116	16–118	37–112
Mean (SD)	84.35 (15.1)	91.63 (15.9)	92.00 (16.5)	90.37 (17.1)	77.56 (15.9)	78.91 (23.7)	83.93 (15.1)	87.28 (16.3)	83.16 (26.7)	91.00 (16.8)
Me	83	89	93	90	80	85	84	89	89	94
25(OH)D >30 ng/mL	**n = 44**									
Min – max	44–115	51–128	42–118	67–125	59–117	28–124	55–116	45–118	36–121	52–125
Mean (SD)	85.70 (18.5)	92.00 (15.4)	93.9 (19.1)	95.09 (13.2)	85.64 (12.8)	83.70 (23.2)	87.57 (14.7)	89.41 (16.1)	91.43 (20.7)	92.07 (16.6)
Me	91.5	92	98	95	85.5	86	89	90.5	97.5	96
25(OH)D Total	**n = 170**									
Min – max	29–115	51–128	42–125	47–125	26–117	18–124	22–116	36–121	16–121	18–125
Mean (SD)	81.19 (17.8)	89.66 (14.8)	90.10 (18.4)	92.83 (15.4)	78.70 (14.8)	80.10 (24.5)	82.63 (17.0)	86.61 ± 16.3	83.58 ± 26.7	86.50 ± 20.1
Me	84	89	88.5	94	79.5	85.5	84.5	88.0	91	92
Kruskal-Wallis statistic	17.94	17.40	13.49	4.311	3.705	3.425	7.696	6.705	9.132	18.44
p value*	0.0005	0.0006	0.0037	0.2297	0.2952	0.3306	0.0527	0.0819	0.0276	0.0004

The examined women had the best average results in terms of visual memory (92.83 ± 15.4) and verbal memory (90.1 ± 18.4 pts.), which correspond with the average level and the weakest results in the processing speed (average – 78.7±14.8 pts. which mean low level).

Table 4 presents the distribution of cognitive functions, with the breakdown into following categories: above, average, low average, low, and very low depending on the concentration range 25(OH) (in percentages).

The very low values of NCI (23.9%), memory (19.6%), verbal memory (17.4%), processing speed (32.6%), executive functioning (39.1%), reaction time (19.6%), complex attention (30.4%), and cognitive flexibility (39.1) were in the majority in women with severe deficiency of 25(OH)D. On the other hand, only 2.2–8.7% participants had memory, verbal memory, and visual memory, which belonged to other categories, i.e., average or low average. The majority of the women with normal 25(OH)D values, in all tested cognitive domains, were classified to the category average (27.3–50%) (Tab. 4).

DISCUSSION

The participants of this study were women in good general condition of health without the active cancer disease, mental diseases including depression in the per-

Table 3. The levels of severity (CNS-VS)

Above >110 pts	High function and high capacity
Average 90–110 pts	Normal function and normal capacity
Low Average 80–90 pts	Sight deficit and sight impairme
Low 70–79 pts	Moderate deficit and impairment possible
Very Low <70 pts	Deficit and impairment likely

imenopausal period, pharmacological and alcohol addictions, and/or diagnosed disease entity with dementia symptoms. The studied women were matched for age, education, weight, environment and they had a similar reproductive period.

Plasma concentration of vitamin D was not related to age, BMI, period of procreation, lipids, and years of education. We only found the significant associations between the severe deficiency of vitamin D with lower NCI scores.

In this study, we used a computerized neurocognitive test battery known as Central Nervous System Vital Signs (CNS-VS), which are designed to detect possible cognitive impairment in the preclinical phase and serve as a marker for referral for further, more specialized, testing. The CNS-VS is suitable for use as a screening tool that measures speed and accuracy of basic mental functions [10]. The battery test includes 7 subtests that assess verbal, visual, and composite memory, executive function, processing speed, psychomotor speed, reaction time, complex attention, and cognitive flexibility. After completion of the tests, the computer program generates a report that includes domain scores derived by factor analysis: memory, psychomotor speed, reaction time, cognitive flexibility, complex attention and the Neurocognition index (NCI) which is an average of the domains scores and reflects the overall performance of the patient. The battery also includes validity indicators for each subtest in order to ensure that the results accurately reflect the patient's cognitive ability [10, 27]. As shown in a study by Gualtieri et al. [10], the battery exhibits good test–retest reliability and that reliability remains unaffected by age or clinical status of patients. The NCI is a summary of the patient's cognitive status, generated by averaging the five domain scores: memory, psychomotor speed, reaction time, attention, and cognitive plasticity.

Table 4. Assessment of cognitive functions in particular groups of 25-hydroxyvitamin D levels [25(OH)D]

Characteristics	Prevalence, %			
Domain	25(OH)D >10 ng/mL	25(OH)D 10–20 ng/mL	25(OH)D 20–30 ng/mL	25(OH)D <30 ng/mL
Neurocognition Index:				
Very Low	23.9	21.6	11.6	11.4
Low	26.1	24.3	16.3	13.6
Low Average	15.2	13.5	23.3	20.5
Average	34.8	37.8	44.2	50.0
Above	0.0	2.7	4.7	4.5
Memory:				
Very Low	19.6	8.1	7.0	4.5
Low	21.7	26.9	14.0	13.7
Low Average	23.9	22.1	23.3	29.5
Average	26.1	35.0	37.2	27.3
Above	8.7	7.9	18.5	25.0
Verbal Memory:				
Very Low	17.4	11.0	9.3	6.8
Low	21.7	24.3	11.6	4.5
Low Average	15.2	5.2	18.6	25.0
Average	30.4	37.7	44.2	36.4
Above	15.3	21.8	16.3	27.3

Characteristics	Prevalence, %			
Domain	25(OH)D >10 ng/mL	25(OH)D 10–20 ng/mL	25(OH)D 20–30 ng/mL	25(OH)D <30 ng/mL
Visual Memory:				
Very Low	8.7	5.4	7.0	4.5
Low	17.4	8.2	20.9	9.1
Low Average	28.3	27.0	18.6	29.5
Average	39.1	48.6	44.2	43.2
Above	6.5	10.8	9.3	13.7
Processing speeding:				
Very Low	32.6	21.6	25.6	9.1
Low	28.3	16.2	18.6	36.4
Low Average	15.2	45.9	23.3	15.9
Average	23.9	10.9	27.8	34.1
Above	0.0	5.4	4.7	4.5
Executive functions:				
Very Low	39.1	21.6	34.9	25.0
Low	10.9	16.2	9.3	15.9
Low Average	10.9	45.9	20.9	13.6
Average	30.4	10.9	20.9	31.9
Above	8.7	5.4	14.0	13.6
Psychomotor speed:				
Very Low	23.9	8.1	14.0	25.0
Low	21.7	10.8	18.6	11.4
Low Average	21.7	29.7	16.3	29.5
Average	28.3	45.9	39.5	29.5
Above	4.4	5.5	11.6	4.6
Reaction time:				
Very Low	19.6	2.7	11.6	9.1
Low	28.3	18.9	7.0	22.7
Low Average	19.5	29.8	27.9	22.7
Average	30.4	43.2	46.5	34.1
Above	2.2	5.4	7.0	11.4
Complex attention:				
Very Low	30.4	27.0	23.3	15.9
Low	26.1	13.5	11.6	6.8
Low Average	13.0	16.2	11.6	13.7
Average	26.1	35.1	37.2	40.9
Above	4.4	8.2	16.3	22.7
Cognitive flexibility				
Very Low	39.1	29.7	20.9	15.9
Low	17.4	16.2	18.6	13.6
Low Average	15.2	16.2	16.3	20.5
Average	21.8	32.5	32.6	31.8
Above	6.5	5.4	11.6	18.2

We revealed that the considerably worse NCI scores were obtained by women with severe deficiency of 25(OH)D in comparison with those with normal levels of vitamin D ($p < 0.001$; Tab. 3). Moreover, women with 25(OH)D levels above 10 ng/mL had similar the global cognitive performance (NCI). In addition, we demonstrated that the cognitive effects of very low 25(OH)D levels were apparent in memory, executive functioning, complex attention, and cognitive flexibility (Tab. 3).

Recent results of studies published on this topic are inconsistent. The difference in findings across the studies might be partially explained by the difference in the cohort population and the definition of impairment or handling of baseline impairment. As reported by Schneider et al. [23], in the study performed in populations of patients in late middle age, significant associations between lower levels of 25(OH)D and lower cognitive test scores at baseline, change in scores over time or dementia risk were not shown. They noticed that many of the previous studies in which significant associations between 25(OH)D levels and dementia were reported, performed in older populations [23].

In contrast, a meta-analysis by Etgen et al. [8] suggested a more than doubled risk of cognitive impairment in patients with vitamin D deficiency among 7.688 participants. In our study, the cognitive impairment was found in postmenopausal women in late middle age (from 50 to 65 years). Llewellyn et al. [13] found an association between very low 25(OH)D levels (<10 ng/m) and risk of cognitive decline as measured by MMSE in a cohort of 858 community-dwelling Italian elderly participants. Similarly, it has been reported that very low 25(OH)D levels (<10 ng/mL) among older women were associated with a higher odds of global cognitive impairment at baseline [25]. The authors also demonstrated that women with 25(OH)D levels between 20 and 30 ng/mL had global cognitive performance similar to that of 25(OH)D sufficient women.

Our results are in general agreement with this data [8, 13, 25], although, in contrast to Slinin et al. [25], we found an association between 25(OH)D levels and the risk of a decline in executive function. A single small placebo controlled trial which incorporated only younger adults did not find a correlation between executive function and vitamin D levels, either [7].

It is worth noting that severe deficiency of 25(OH)D did not cause the dysfunction in all domains of neurocognitive. However, we found no dependence between 25(OH)D levels and decline in visual memory, processing speed, executive functioning, psychomotor speed, and reaction time (Tab. 3). Thus, it is possible that vitamin D has a direct effect on decline in some cognitive domains but not in all. Recently, Annweiler et al. [2] and Schneider et al. [23] have presented a review of studies of memory and executive dysfunction in relationship to vitamin D blood levels, published in the last years. The meta-analysis provided evidence that low vitamin D is associated in adults with impaired episodic memory and executive dysfunction, especially mental shifting, informational updating and processing speed. Individuals with high vitamin D levels exhibited better executive function, especially in information updating and processing speed, all very important as relates to reasoning, judgment and decision making. Moreover, it has been shown that low vitamin D levels precede a decline in executive function and treating low levels of vitamin D were associated with improved executive function [1]. Buell et al. [6] also found a positive association between serum 25(OH)D and primary measures of executive functioning and attention processing speed, but not memory (as assessed by word list learning and logical learning) in elderly subjects (76% of which were women).

Vitamin D might have a neuroprotective effect by preventing vascular dementia [6]. On the other hand, it has been demonstrated that vitamin D is a cardiovascular risk factor and promote the onset of other vascular risk factors such as atherosclerosis and hypertension, which leads to impaired executive dysfunction [15].

In our study, we did not find an association between estradiol levels and cognitive impairment. The results of extensive research, using a variety of learning and memory tasks, show that estradiol generally enhances cognition [14]. It has been demonstrated that young women with high levels of salivary estradiol (7.3 pmol/L) had fewer working memory errors on a spatial working memory task than when their levels of estradiol were low (2.2 pmol/L). Moreover, Bagger et al. [3] found that treatment with estradiol for 2 to 3 years around menopause decreased the risk of cognitive impairments 5–15 years later by 64% as compared with those who received a placebo. The difference in findings across the studies might be partially explained by the differences in the studied population, age of participants, and the definition of cognition impairment.

We showed that genotypes APOE ε3/ε4 or ε4/ε4 were most common (19.6%; 15.2%, respectively) in women with 25(OH)D severe deficiency, which had the weakest average results in terms of NCI value in comparison with other groups of postmenopausal women. Mean results in NCI, which express the global cognitive performance in these women, were 71.52 ± 18.1 pts, i.e. low evaluations in NCI, while in the remaining women, mean values were about 85 pts. These findings are in agreement with those published by Bojar et al. [5] in postmenopausal women, indicating that the considerably poorer NCI was obtained by women with ε3/ε4 and ε4/ε4 polymorphisms. In contrast, Huebbe el al. [12] showed experimental and epidemiological evidence that the APOE ε4 allele is associated with higher serum vitamin D [25(OH)D] level. However, these results were obtained in men and women population with mild insufficiency of vitamin D, while our results concern postmenopausal women with severe deficiency this vitamin.

The ε4 allele and low serum vitamin D levels are known susceptibility factors for CVD [9]. Therefore, a possible reason for the association of the apoE4 genotype and cognitive impairment is that apoE4 genotype is related to vascular disease, which is known to cause cognitive impairment [17]. In addition, hypertension is the primary and most important manifesting symptom of hypertensive vascular disease. In the current study, the prevalence of hypertension (blood pressure >140/90 mmHg) in women with very low vitamin D levels was small (21.7%) (Tab. 1), which may suggest a lack of vascular disease in these women.

The strengths of the study is that women were matched for age, education, environment and a standard analytical method was employed to quantify 25(OH)D levels, but it also had several limitations. Only one baseline measurement of vitamin D was available for analysis. Therefore, the absolute effect size of the association between baseline vitamin D level and cognitive disorders requires further detailed research. Moreover, the restricted number of subjects with severe 25(OH) D deficiency might have influenced our findings. The restricted number of subjects with severe 25(OH)D deficiency might have influenced our findings. Further studies are necessary to replicate our findings and extend them to more diverse populations, with a significantly increased number of participants. It would be useful to conduct prospective studies in postmenopausal women to investigate the association between vitamin D concentrations and vascular disorders and neuroimaging abnormalities.

In conclusion, the severe deficiency of 25(OH)D vitamin was related with a greater likelihood of cognitive impairment and risk of cognitive decline in the field of memory, executive functioning, complex attention and cognitive flexibility in postmenopausal women with no dementia. Our findings support the hypothesis that vitamin D may be neuroprotective in these women and that "sufficiency" in the context of cognition risk may be in the range of 20 ng/ml.

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