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Lower 25-hydroxyvitamin D is associated with severer white matter hyperintensity and cognitive function in patients with non-disabling ischemic cerebrovascular events

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ABSTRACT

Objectives: This study aimed to investigate the potential correlations among serum 25-hydroxyvitamin D [25(OH)D] levels, white matter hyperintensity (WMH) and cognitive function in patients with non-disabling ischemic cerebrovascular events (NICE).

Methods: This was a prospective investigation of 160 NICE patients with age of 40 years or older. Cognitive function was evaluated by the Montreal Cognitive Assessment (MoCA). White matter lesions were evaluated by WMH using Fazekas scores. Spearman correlation analysis and linear regression models were used to identify the associations between serum 25(OH)D levels and cognitive function. Binary logistic regression analysis models were used to evaluate the predictable value of serum 25(OH)D levels and WMH for cognitive impairment.

Results: Patients with inadequate 25(OH)D levels had lower MoCA score (P=0.008), and a higher proportion of severe WMH (P=0.043). Spearman correlation analysis demonstrated that serum 25(OH)D concentrations were positively associated with MoCA score (r_s=0.185, P=0.019) while negatively related to the proportion of severe WMH (sWMH) (r_s=-0.166, P=0.036).The association between 25(OH)D concentrations and MoCA score remained significant in linear regression (adjusted β=0.012, 95%CI:0.001-0.203).Adjusted binary logistic regression analysis showed that the odds ratio of cognitive impairment with insufficient 25(OH)D concentration was 5.038 (95%CI:1.154-21.988) compared with the sufficient group and the sWMH (OR=2.728, 95%CI:1.230-6.051) was identified as an independent risk factor for cognitive decline in NICE patients.

Conclusion: Serum 25(OH)D levels and white matter lesions were independently and significantly associated with cognitive impairment in NICE patients.

Introduction

Vascular cognitive impairment (VCI) is the second most common type of cognitive impairment, referring to the entire spectrum of cognitive impairment contributed by cerebrovascular pathology[1]. Due to the progressively higher incidence of stroke and cerebrovascular diseases, VCI, including post-stroke cognitive impairment are the leading causes of disability epidemic worldwide[2-3]. White matter lesions, manifested as white matter hyperintensities (WMH) on T2-weighted magnetic resonance imaging (MRI) scans, are one of the most common pathological characteristics of VCI[4]. Despite the high prevalence and disability of VCI, no licensed therapeutic measures have been approved, the main intervention is preventing and treating risk factors[1].

Consisting of transient ischemic attack (TIA), minor ischemic stroke (NIHSS score≤5 or 3), and unimpaired stroke with rapid remission, non-disabling ischemic cerebrovascular events (NICE) refers to ischemic

Abbreviation: 25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; IQCODE, the Informant Questionnaire on Cognitive Decline in the Elderly; NCI, no cognitive impairment; NICE, non-disabling ischemic cerebrovascular events; VCI, vascular cognitive impairment; WMH, white matter hyperintensity.

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cerebrovascular disease with no residual neurological disability[5] and has a considerable high recurrence. A large-scale clinical trial, the CHANCE study has reported that, even received dual antiplatelets within 24 h, the recurrence rates of NICE patients within 3 months were still as high as 9.4%[6]. Besides, our recent study has found that more than half of NICE patients has cognitive disorders at the time of admission, and nearly half of patients presented with severe WMH in MRI[7]. Therefore, despite the mild symptoms at the time of onset, NICE is still a disease that should not be ignored and NICE patients are at a very high risk of disability progression and development of VCI.

Recent years, there has been controversy over dietary and vitamin alterations for cognition. In order to dig out possible primary preventions, investigators have dedicated to identify nutritional factors associated with cognitive impairment and dementia. In this regard, as a safe and easily available nutritional supplement, growing evidence indicates that vitamin D may play a potential role in maintaining favorable cognitive function. Numerous studies have suggested the beneficial effect of higher vitamin D concentrations in preventing or delaying dementia progression[8-11]. Besides, the association between vitamin D deficiency and WMH was found in the chronic cerebral small vessel disease[12]. So, is vitamin D deficiency prevalent in patients with NICE? Is vitamin D deficiency associated with severer WMH and worse cognition of NICE patients? Could vitamin D be a potential intervention to prevent VCI in such patients? This study was aimed to answer the above specified questions.

Materials and methods

Study design and participants

This was a prospective study with cross-sectional analysis, enrolling NICE patients from the First Affiliated Hospital of Naval Medical University between January 2021 and October 2022. This study was approved and carried out in accordance with the recommendations of the Ethics Committee of Changhai Hospital, Navy Medical University (CHEC2021-102). All participants signed the written informed consent. Patients were eligible if they met the following criteria[6]: a) age of 40 years or older; b) first episode of acute stroke with admission within one week of onset; c) National Institute of Health Stroke Scale Score (NIHSS) $\leq$3 at admission; d) neurological deficits with none or mild adverse impact on daily life.

Notable exclusion criteria included: a) missing vitamin D records; b) unable to complete the psychological assessment due to severe aphasia, dysarthria or audio and vision impairment; c) presence of cognitive impairment other than cerebrovascular diseases; d) a history of major depression or other mental disorders; e) a history of any central nervous system disease; f) inability to undergo MRI examination; g) failed to sign the informed consent form or refuse participation.

Serum vitamin D measurement and status determination

Fresh drawn venous blood was collected from patients at recruitment and the serum vitamin D level was tested within 24 h of hospitalization at the baseline. The serum of 25-hydroxyvitamin D (25(OH)D) level, the preferred biomarker of vitamin D nutrition status[13], was measured with an electrochemiluminescence protein binding assay (COBAS e411; Roche Diagnostics, Germany) in the biochemistry department of our hospital. The intra-assay coefficient of variation was 7.8-10.7%. All the measurements were performed blinded to the clinical data.

Consistent with relevant reference populations[14], the level of 25(OH)D was stratified into three categories: >30 ng/mL as sufficiency, 20–30 ng/mL as insufficiency, < 20 ng/mL as deficiency.

MRI acquisition and imaging analysis

Cranial MRI was performed in all enrolled patients with a standard MRI protocol including T1-weighted (T1WI), T2-weighted (T2WI), and T2-weighted fluid- attenuated inversion recovery (T2WI-FLAIR) sequences as previously depicted[7].

White matter hyperintensities (WMH) were defined as hyperintense changes on T2WI and FLAIR with no corresponding T1 abnormality. By using the Fazekas scale[15], the extent of WMH was graded into two levels[16]: white matter in deep and/or periventricular region with Fazekas scores of 0 and 1 was defined as mild WMH (mWMH), while the one with Fazekas scores of 2 and 3 was defined as severe WMH (sWMH). All imaging results were independently evaluated by two trained neurologists who were blinded to the clinical information.

Neuropsychological assessment

The neuropsychological assessment was performed by experienced neurologists blinded to the results of other clinical and laboratory information within two weeks of NICE occurrence[17]. In terms of cognitive function, the patients' cognition status before NICE was determined by the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) and those with an IQCODE score more than 52 were excluded to eliminate the chronic impact of cognitive impairment[18]. The global cognitive function was evaluated using the Montreal Cognitive Assessment (MoCA) scale. According to previous studies based on Chinese population, the patients were divided into two groups when the cut-off score is 24[7]: the VCI group was defined as MoCA scores less than 24, while patients with no cognitive impairment (NCI) was defined as MoCA scores of 24 or more. Additionally, different scales were used to evaluate the cognitive domains[19] including the Auditory Verbal Learning Test (AVLT) for short and long-term memory, Trail Making Test A (TMT-A) and Symbol Digit Modalities Test (SDMT) for attention, Trail Making Test B (TMT-B) for executive function and Verbal Fluency Test (VFT) for language function.

Furthermore, depressive symptoms were measured using the 24-item Hamilton Depression Scale (HAMD). And the Apathy Evaluation Scale-Clinician Version (AES-C) was used to assess apathy[20]. Higher scores of these instruments reflect worse psychological symptoms.

Statistical analysis

All statistical analyses were performed using SPSS (version 26.0, IBM Corporation, Armonk, NY, USA). The Kolmogorov–Smirnov test was conducted to figure out whether the variable distributions were normal or not. Based on the distribution type, continuous variables were expressed as mean $\pm$ standard deviation or as the median (interquartile range, IQR), and comparisons between the VCI and NCI groups were used by Student's t-test and Mann-Whitney U-test, respectively. Categorical variables were presented as frequency, and Chi-square test was performed to compare between groups. To compare the differences among three vitamin D levels, one-way analysis of variance (ANOVA) or the Kruskal-Wallis test was used for continuous variables and Pearson's Chi-square test or Fisher's exact test was performed for categorical variables.

Furthermore, Spearman correlation was used to estimate the relationship between serum 25(OH)D level and neuropsychological performances along with WMH scores in patients with NICE. A binary logistic regression was used to identify independent risk factors for cognitive function after acute ischemic stroke events. Covariates were selected based on clinical factors and confounding effects including age, sex, BMI, education years, diabetes, hypertension and hyperlipidemia. Statistical significance was defined as a two-tailed $P < 0.05$.

Results

Characteristics of patients according to cognitive function

In total, 263 NICE patients were originally enrolled in this study. And

160 eligible patients (87 males and 73 females, with mean age at 63.4 years) were consequently included according to inclusion criteria (Fig. 1).

As revealed by Table 1, there is no differences of age, male percentage, BMI, history of hypertension, diabetes mellitus, hyperlipidemia, current smoking or drinking between the VCI and NCI groups (all *Ps*> 0.05). VCI group had lower education years ($P < 0.001$), yet worse cognitive performance on all scales tested (all *Ps*< 0.01) than those of NCI group. Besides, patients from VCI group had higher HAMD and AES-C scores, indicating poorer psychological conditions. Regarding the laboratory tests, compared with NCI group, VCI group had lower levels of LDL and TC, while other parameters, including parathyroid hormone, calcium, phosphorus, TG, HDL, creatinine, GFR showed no significant differences (all *Ps*> 0.05). What's more, vitamin D insufficiency and deficiency were prevalent in NICE patients (88.1%). It is not surprising to find that VCI group had lower serum 25(OH)D level than that of NCI group (19.5 ng/mL (IQR, 15.9-23.2) vs. 21.6 ng/mL (IQR, 16.1-28.7); $P < 0.001$) and the proportion of patients with sufficient serum vitamin D level was significantly higher in the NCI group (20.0% vs. 3.8%, P=0.006, Table1).

Characteristics of patients according to serum vitamin D levels

Patients were divided into three subgroups according to serum 25 (OH)D concentrations, and comparable percentages of patients fell under each of the three levels. As shown by Table 2, parathyroid hormone (P=0.043) gradually decreased when vitamin D level increased from the lowest to the highest, and there were tendencies of higher MoCA score, SDMT score, as well as VFT score (P=0.008, 0.020, 0.030, and 0.043, respectively).. The prevalence of patients with sWMH appeared to decrease with increasing vitamin D concentrations, although the trend was not statistically significant (P=0.072). When sub-grouped these NICE patients into sufficient and insufficient vitamin D levels as depicted in Fig. 2, patients with insufficient and deficient serum vitamin D levels had a higher percentage of sWMH(P =0.043, Fig. 2). No other baseline characteristics were found significantly different among the three groups.

Correlations among serum vitamin D level, WMH with neuropsychological scores

As revealed in Table 3, Spearman correlation analysis showed that the serum 25(OH)D level was positively correlated with MoCA score (r_s=0.185, P=0.019), SDMT score (r_s=0.173, P=0.035) and VFT score (r_s=0.173, P=0.044; Table 3), while negatively related to AES-C score (r_s=-0.202, P=0.011). Interestingly, no relationship was found between HAMD and vitamin D level. Besides, patients with lower serum 25(OH)D levels had a significantly higher proportion of sWMH (r_s=-0.166, P=0.036, Fig. 3). Furthermore, after adjusting for age, sex, education and BMI (Table 4, model 1), linear regression analysis confirmed that serum 25(OH)D level had a positive association with MoCA score (B=0.107, CI=0.009, 0.205; P=0.033), a negative correlation with ASE-

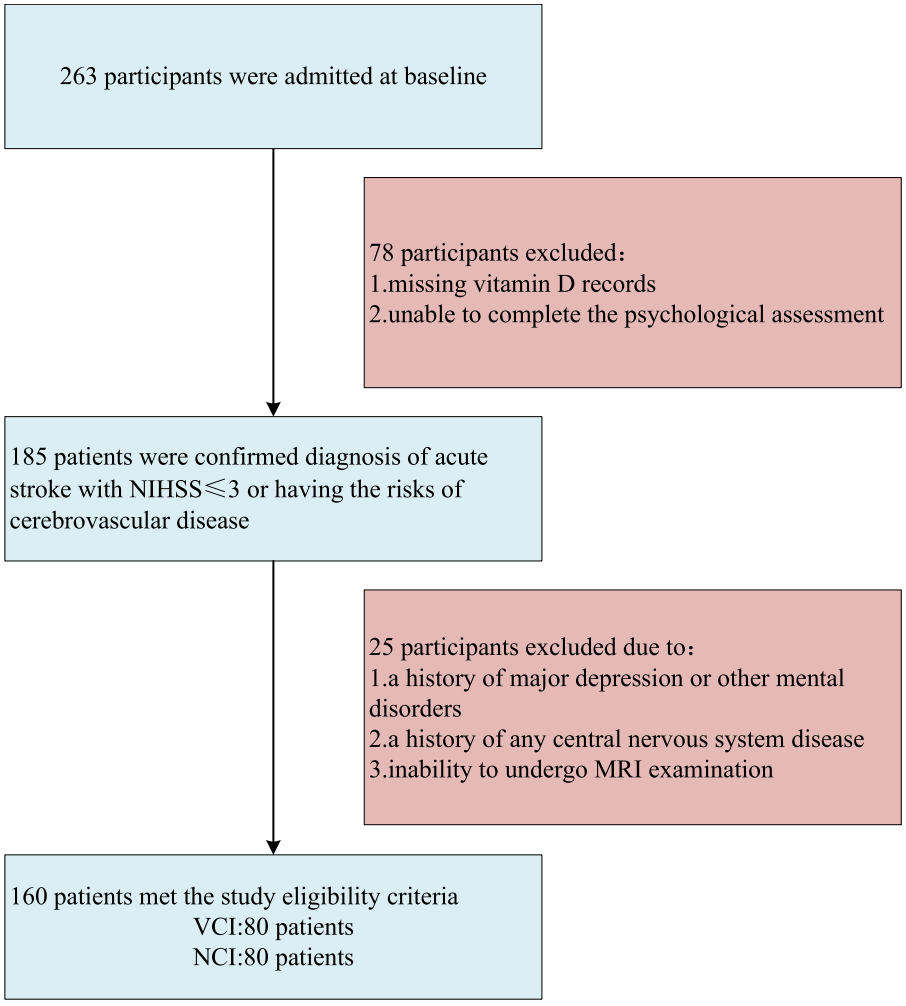

Fig. 1. Schematic representation of patient enrollment. NCI, no cognitive impairment; NIHSS, National Institute of Health stroke scale; MRI, magnetic resonance imaging; VCI, vascular cognitive impairment.

Table 1
Demographic and clinical characteristics of NICE patients, stratified by cognitive function.

Variables[1]	NCI[2]	VCI[2]	P-value
Participants, n	80	80	
Age (y, mean ± SD)	62.7±9.0	64.1±8.8	0.344
Gender (male, n,%)	46(57.5%)	41(51.2%)	0.427
BMI (kg/m^2, mean ± SD)	24.5±3.4	25.0±3.3	0.315
Education (y)	12.0(9.0-15.0)	9.0(6.0-12.0)	**<0.001**
History of hypertension (n, %)	56(70.0%)	55(68.8%)	0.864
History of diabetes (n, %)	23(28.7%)	24(30.0%)	0.862
History of hyperlipidemia (n, %)	25(31.3%)	17(21.3%)	0.151
Smoking (n, %)	38(47.5%)	33(41.3%)	0.426
Drinking (n, %)	22(27.8%)	25(32.1%)	0.565
NIHSS on admission (score)	2.0(1.0-3.0)	2.0(1.0-3.0)	0.645
Laboratory parameters			
25(OH)D concentration (ng/mL)	21.6(16.1-28.7)	19.5(15.9-23.2)	**0.047**
25(OH)D			**0.006**
<20 ng/mL (n, %)	35(43.8%)	43(53.8%)	
20-30 ng/mL (n, %)	29(36.3%)	34(42.5%)	0.252
≥30 ng/mL (n, %)	16(20.0%)	3(3.8%)	0.427
Parathyroid Hormone (pg/mL)	41.8(32.7-55.9)	45.8(35.9-58.7)	
Calcium (mmol/L, mean ± SD)	2.3±0.1	2.2±0.1	
Phosphorus (mmol/L)	1.2(1.1-1.3)	1.2(1.1-1.3)	0.774
TG (mmol/L)	1.5(1.0-2.1)	1.2(1.0-1.9)	0.246
TC (mmol/L, mean ± SD)	4.6±1.1	4.3±0.9	**0.048**
HDL-C (mmol/L)	1.2(1.1-1.5)	1.2(1.1-1.5)	0.978
LDL-C (mmol/L, mean ± SD)	2.7±1.0	2.4±0.8	0.021
Creatinine (μmol/L)	75.0(61.0-87.0)	69.0(57.3-80.8)	0.099
GFR (mL/min, mean ± SD)	94.3±25.7	97.2±21.7	0.459
Neuropsychological performance			
MoCA (score)	26.0(25.0-27.0)	20.0(17.0-22.0)	**<0.001**
AVLT			
N1+N2+N3 (score)	17.0(14.3-20.0)	13.0(10.0-16.0)	**<0.001**
N4 (score)	5.0(4.0-7.0)	3.0(2.0-5.0)	**<0.001**
N5 (score)	5.0(3.0-7.0)	3.0(1.3-5.0)	**<0.001**
TMT-A (s)	59.0(46.8-69.3)	72.5(56.3-126.0)	**<0.001**
TMT-B (s)	135.0(103.0-180.0)	165.0(123.0-208.8)	**0.002**
SDMT (score, mean ± SD)	33.2±11.1	23.2±11.6	**<0.001**
VFT (score)	17.0(14.0-21.0)	13.0(11.0-17.0)	**<0.001**
HAMD (score, mean ± SD)	4.9±6.1	7.3±6.9	**0.023**
AES-C (score)	24.5(20.3-30.0)	30.0(24.3-36.0)	**<0.001**
WMH, Severe (n, %)	28(35.0%)	49(61.3%)	**0.001**

[1]AES-C, Apathy Evaluation Scale-Clinician Version; BMI, body mass index; GFR, glomerular filtration rate; HAMD, Hamilton Depression Scale; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; 25(OH)D, 25-hydroxyvitamin D; MoCA, Montreal Cognitive Assessment; NCI, no cognitive impairment; NIHSS, national institutes of health stroke scale; SDMT, Symbol Digit Modalities Test; TC, total cholesterol; TG, triglyceride; TMT-A, Trail Making Test A; TMT-B, Trail Making Test B; VCI, vascular cognitive impairment; VFT, Verbal Fluency Test; WMH, white matter hyperintensity.
[2]Normally distributed continuous variables were presented as mean ± SD and compared using t-tests. Nonnormally distributed continuous variables were presented as median (IQR) and compared using rank-sum tests. Categorical variables were presented as number (percentage) and compared using the chi-square test.

C score (B=-0.257, CI=-0.009, 0.063; *P*=0.010), but not with SDMT score (*P*=0.199) and VFT score(*P*=0.179). Associations remained the same after additionally adjusting for vascular risk factors, including the history of hypertension, hypercholesterolemia, diabetes, smoking and drinking (Table 4, model 2).

Association of serum vitamin D levels and WMH with diagnosis of VCI in patients

The results of logistic regression analyses were shown in Table 5. It was found that risk of VCI increased with reduced level of serum 25(OH)

Table 2
Baseline characteristics of NICE patients according to serum vitamin D levels.

Variables	25(OH)D concentration <20 ng/ml (deficient)	20-30 ng/ml (insufficient)	≥30 ng/ml (sufficient)	P-value
Participants, n	78	63	19	
Age (y, mean ± SD)	64.5±8.6	62.0±9.6	63.2±7.4	0.252
Gender (male, n,%)	36(46.2%)	38(60.3%)	13(68.4%)	0.104
BMI (kg/m^2, mean ± SD)	24.8±3.7	24.9±2.9	24.3±3.6	0.803
Education years (y)	9.5(9.0-12.0)	9.0(9.0-13.0)	13.0(9.0-15.0)	0.133
History of hypertension (n, %)	55(70.5%)	44(69.8%)	12(63.2%)	0.819
History of diabetes (n, %)	26(33.3%)	17(27.0%)	4(21.1%)	0.497
History of hyperlipidemia (n, %)	20(25.6%)	16(25.4%)	6(31.6%)	0.853
Smoking (n, %)	31(39.7%)	31(49.2%)	9(47.4%)	0.511
Drinking (n, %)	24(31.6%)	16(25.4%)	7(38.9%)	0.495
NIHSS on admission (score)	2.0(1.0-3.0)	2.0(1.0-3.0)	1.0(0.0-2.0)	0.113
Laboratory parameters				
25(OH)D concentration (ng/mL)	15.9(13.9-18.2)	24.1(22.1-26.1)	32.2(31.4-36.8)	**<0.001**
Parathyroid Hormone (pg/mL)	47.6(34.5-62.7)	41.4(36.0-52.1)	35.8(26.1-53.6)	**0.043**
Calcium (mmol/L, mean ± SD)	2.2±0.1	2.2±0.1	2.2±0.1	0.237
Phosphorus (mmol/L)	1.3(1.1-1.4)	1.2(1.1-1.3)	1.3(1.1-1.3)	0.354
TG (mmol/L)	1.5(1.1-2.0)	1.4(1.0-2.1)	1.0(0.9-1.7)	0.111
TC (mmol/L, mean ± SD)	4.5±1.2	4.4±1.0	4.5±0.7	0.732
HDL (mmol/L)	1.2(1.0-1.5)	1.2(1.1-1.4)	1.4(1.1-1.7)	0.159
LDL (mmol/L, mean ± SD)	2.6±1.0	2.4±0.8	2.7±0.7	0.487
Creatinine (μmol/L)	72.0(56.5-84.5)	70.0(62.0-83.0)	72.0(65.0-84.0)	0.720
GFR (mL/min, mean ± SD)	92.9±27.7	98.8±19.0	96.9±19.1	0.360
Neuropsychological performance				
MoCA (score)	23.0(19.8-25.0)	23.0(20.0-26.0)	26.0(24.0-26.0)	**0.008**
AVLT				
N1+N2+N3 (score)	15.0(11.0-18.0)	16.0(12.5-18.0)	16.0(12.8-18.3)	0.657
N4 (score)	4.0(2.0-7.0)	5.0(3.0-6.0)	5.0(3.8-7.0)	0.452
N5 (score)	3.5(2.0-6.0)	4.0(3.0-6.0)	4.0(3.0-7.0)	0.625
TMT-A (s)	68.0(50.0-94.0)	64.0(50.0-87.0)	58.5(38.0-65.8)	0.060
TMT-B (s)	150.0 (115.3-202.8)	148.0(117.0-190.0)	123.5 (102.8-166.3)	0.317
SDMT (score)	25.9±11.9	29.1±12.8	34.7±10.3	**0.020**
VFT (score)	14.0(12.0-18.0)	16.0(13.0-20.0)	18.0(12.8-20.8)	**0.030**
HAMD (score)	5.0(1.0-8.0)	4.0(1.0-8.0)	4.0(1.0-7.0)	0.552
AES-C (score)	28.0(23.0-35.0)	27.0(21.0-33.3)	24.0(20.0-30.0)	0.133
WMH, Severe (n, %)	43(55.1%)	29(46.0%)	5(26.3%)	0.072

[1]AES-C, Apathy Evaluation Scale-Clinician Version; BMI, body mass index; GFR, glomerular filtration rate; HAMD, Hamilton Depression Scale; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; 25(OH)D, 25-hydroxyvitamin D; MoCA, Montreal Cognitive Assessment; NCI, no cognitive impairment; NIHSS, national institutes of health stroke scale; SDMT, Symbol Digit Modalities Test; TC, total cholesterol; TG, triglyceride; TMT-A, Trail Making Test A; TMT-B, Trail Making Test B; VCI, vascular cognitive impairment; VFT, Verbal Fluency Test; WMH, white matter hyperintensity.
[2]Normally distributed continuous variables were presented as mean ± SD and

compared using t-tests. Nonnormally distributed continuous variables were presented as median (IQR) and compared using rank-sum tests. Categorical variables were presented as number (percentage) and compared using the chi-square test.

D (*P*-trend =0.017). The odds ratios (ORs) calculated for risks of VCI in patients with deficient and insufficient serum 25(OH)D concentrations were 6.552 (95% CI=1.766-24.317, *P*=0.005) and 6.253 (95% CI=1.625-23.617, *P*=0.017) relative to patients with a sufficient serum 25(OH)D level. After adjusting for covariates in the univariate analyses, including age, sex, education years, BMI and vascular risk factors, the negative correlation trend was no longer existed (*P*-trend=0.122), but the VCI risks in patients with deficient (OR=5.153, 95%CI=1.206-22.021, *P*=0.027) and insufficient (OR=5.834, 95%CI=1.342-25.356, *P*=0.019) 25(OH)D concentrations were still higher than that of patients with a sufficient one. Furthermore, in order to exclude the effect of psychological status on cognition, the covariates mentioned above, the HAMD score and AES-C score were further collectively adjusted, which showed that the association was not existed but the patients with an insufficient serum 25(OH)D level still had a 5.038 (95%CI=1.154-21.988) higher risk of VCI relative to patients with sufficient one.

When we consider WMH, the results are surprisingly similar. The severe WMHs could also be identified as a risk factor of cognitive impairment for NICE patients (OR=2.935, 95%CI=1.543-5.584, P=0.001). After adjusting for the covariates from Model 1 and Model 2, the associations of severe WMHs with VCI were not attenuated (OR=3.286, 95%CI=1.517-7.118, *P*=0.003; OR=2.728, 95%CI=1.230-6.051, *P*=0.014).

Discussion

In this study, we investigated the association among serum vitamin D levels, WMH and neuropsychological performances in patients with non-disabling ischemic cerebrovascular events (NICE). Our findings revealed that:1) vitamin D inadequacy is prevalent in patients with NICE; 2) vitamin D was positively associated with MOCA score, while inversely related to the proportion of severe WMH and AES-C score; 3) VCI risks gradually increased when vitamin D level decreased from the highest to the lowest, and there were tendencies of severer WMH. Indeed, a total of 88.1% of NICE patients presented with vitamin D inadequacy and the prevalence of vitamin D insufficient was 39.4%, whilst 48.7% were deficiency, which was in accordance with previous studies[21-23]. This may be due to the lack of sunlight exposure, limited outdoor activities and malnutrition after stroke[24].

Vitamin D, a lipid-soluble vitamin regarded as a neurosteroid hormone, has been proved to play an important role in brain health and

Table 3
Spearman correlation analyses of 25(OH)D concentration with neuropsychological tests.

Parameters[1]	r_s	P value
MoCA	0.185	**0.019**
AVLT		
N1+N2+N3	0.059	0.489
N4	0.073	0.391
N5	0.089	0.281
TMT-A	-0.160	0.050
TMT-B	-0.075	0.379
SDMT	0.173	**0.035**
VFT	0.173	**0.044**
HAMD	-0.080	0.317
AES-C	-0.202	**0.011**

[1]AES-C, Apathy Evaluation Scale-Clinician Version; HAMD, Hamilton Depression Scale; MoCA, Montreal Cognitive Assessment; SDMT, Symbol Digit Modalities Test; TMT-A, Trail Making Test A; TMT-B, Trail Making Test B; VFT, Verbal Fluency Test.
[2]r_s, Spearman correlation coefficient.

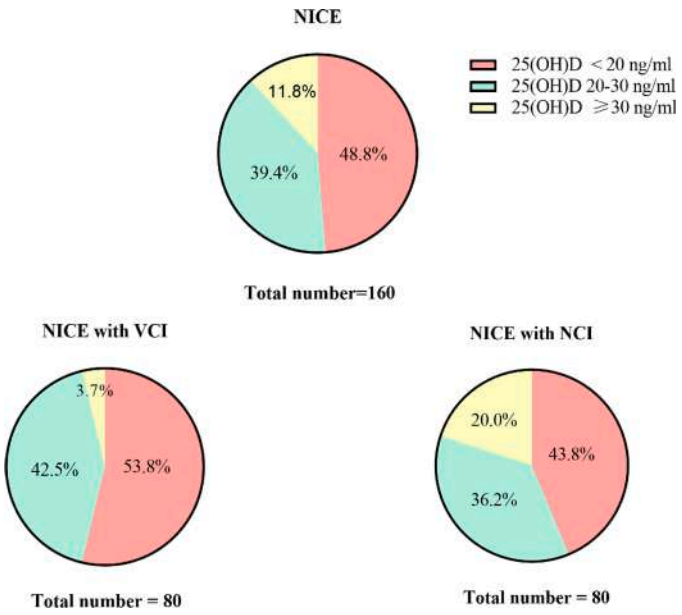

Fig. 3. 25-hydroxyvitamin D status in NICE patients. 25(OH)D, 25-hydroxyvitamin D; NICE, non-disabling ischemic cerebrovascular events (n = 160); NCI, no cognitive impairment (n = 80); VCI, vascular cognitive impairment (n = 80).

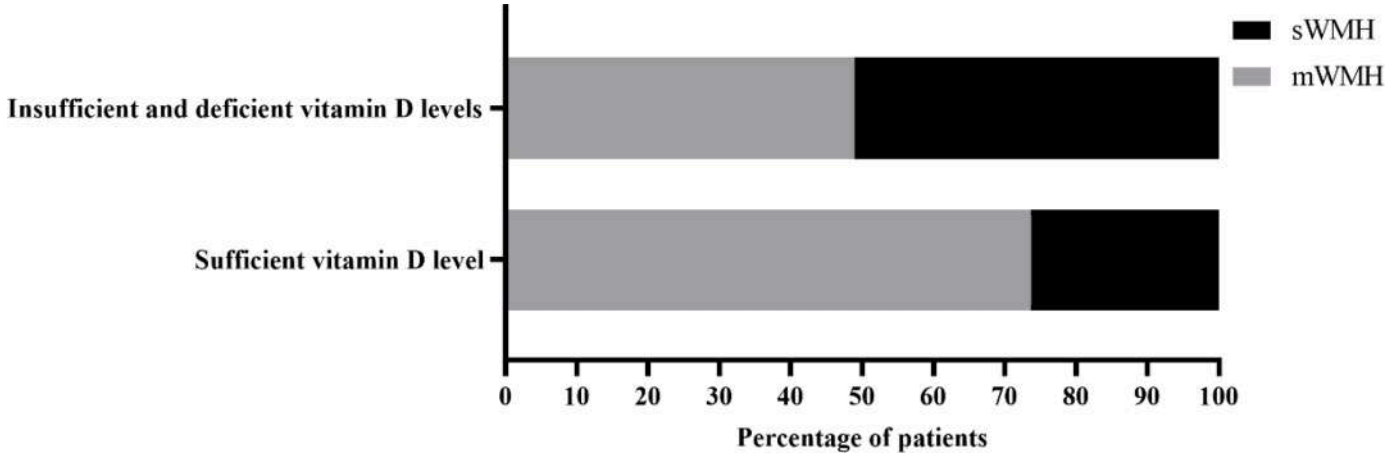

Fig. 2. Proportion of people between mWMH and sWMH in NICE patients with different serum 25(OH)D levels. 25(OH)D, 25-hydroxyvitamin D; NICE, non-disabling ischemic cerebrovascular events; mWMH, mild white matter hyperintensity; sWMH, severe white matter hyperintensity.

Table 4
Regression analysis to identify associations between 25(OH)D concentration and neuropsychological tests.

Parameters[1]		Linear regression analysis	Multivariable regression analysis Model 1[2]	Model 2[3]
MoCA	B (95% CI)	0.161 (0.058,0.263)	0.107 (0.009,0.205)	0.102 (0.001,0.203)
	P	**0.002**	**0.033**	**0.048**
SDMT	B (95% CI)	0.338 (0.053,0.623)	0.173 (-0.092,0.439)	0.136 (-0.123,0.394)
	P	**0.020**	0.199	0.302
VFT	B (95% CI)	0.136 (0.015,0.257)	0.079 (-0.037,0.195)	0.085 (-0.036,0.206)
	P	**0.028**	0.179	0.167
AES-C	B (95% CI)	-0.330(-0.524, -0.136)	-0.257(-0.451, -0.063)	-0.217(-0.414, -0.020)
	P	**0.001**	**0.010**	**0.031**

[1]AES-C, Apathy Evaluation Scale-Clinician Version; MoCA, Montreal Cognitive Assessment; SDMT, Symbol Digit Modalities Test; VFT, Verbal Fluency Test.
[2]Model 1: adjusted fort age, sex, education and BMI
[3]Model 2:adjusted for age, sex, education, BMI and risk factors (hypertension, hypercholesterolemia, diabetes, smoking and drinking)

Table 5
Logistic regression analysis for variables with risk of vascular cognitive impairment.

	OR (95%CI) Serum 25(OH)D levels <20ng/ml	20-30ng/ml	≥30ng/ml	WMH[1] Severe	Mild
Unadjusted	6.552 (1.766-24.317)	6.253 (1.656-23.617)	1.000 (ref)	2.935 (1.543-5.584)	1.000 (ref)
P-value	**0.005**	**0.007**		**0.001**	
P-value for trend		**0.017**			
Model 1	5.153 (1.206-22.021)	5.834 (1.342-25.356)	1.000 (ref)	3.286 (1.517-7.118)	1.000 (ref)
P-value	**0.027**	**0.019**		**0.003**	
P-value for trend		0.122			
Model 2	3.805 (0.886-16.340)	5.038 (1.154-21.988)	1.000 (ref)	2.728 (1.230-6.051)	1.000 (ref)
P-value	0.072	**0.031**		**0.014**	
P-value for trend		0.283			

[1]WMH, white matter hyperintensity.
[2]Model 1: age, sex, education, BMI and risk factors (hypertension, hypercholesterolemia, diabetes, smoking and drinking).
[3]Model 2: Model 1 + HAMD score and AES-C score.

cognitive function[25]. However, the correlation between vitamin D and VCI is still uncertain and very few studies are available in this field. Our findings indicated that VCI individuals had lower serum vitamin D level and the concentration of 25(OH)D was positively associated with cognitive function. This result was consistent with previous retrospective studies which revealed that lower level of serum 25(OH)D was associated with worse cognition in patients with ischemic stroke[26,27]. Meanwhile, logistic regression models demonstrated that, independent of established risk factors, higher vitamin D level was identified as a protective factor for cognitive decline in NICE patients. A prospective study by Chen et al., once reported that vitamin D deficiency was associated with a higher incidence of cognitive impairment in patients with acute ischemic stroke at 1-month follow-up, suggesting that the serum 25(OH)D level might be a potential indicator and intervention target for post-stroke cognitive impairment[28]. However, because of the short-term follow-up duration, further longitudinal study is necessary to assess how vitamin D levels have influence on VCI. Luckily, some basic researches have provided us with preliminary clues. For example, vitamin D exerts various effects on cerebrovascular physiological processes[29]. Thus, vitamin D deficiency may contribute to VCI by influencing cerebrovascular regulation and brain homeostasis, that is, disrupting the regulation of tissue factors, impairing the biosynthesis of neurotransmitters and retarding detoxification pathways of the brain[30]. Besides, vitamin D deficiency may interfere with the regulation of cerebral blood flow[31], decrease endothelial relaxation capacity[32] and compromise cerebrovascular adaptation to ischemic events[33], which may increase the risk of cognitive impairment after stroke. What's more, accumulating evidence suggests that vitamin D deficiency is linked with inflammation, and our previous studies had identified both of central and peripheral inflammation as a strong pathology of VCI[34]. Therefore, we support this idea that NICE patients with low levels of vitamin D are more likely to develop VCI through multiple pathways.

Our results also demonstrated that severe WMH was a risk factor for VCI in NICE patients and sWMH were more prevalent in NICE patients with inadequate concentrations of serum 25(OH)D. Similar results were reported that vitamin D levels was inversely associated with the severity of WMH in patients with mild cognitive impairment, Alzheimer's disease[35].

Despite the lack of definitive evidence to date, several explanations may account for this. First, vitamin D is an essential nutrient for the proliferation and differentiation of oligodendrocyte, who wraps around neuronal axons to form myelin sheaths[36]. Our recent study has firmly confirmed that, vitamin D receptor (VDR), which is broadly present in both neurons and glial cells, is indispensable for remyelination after VCI [unpublished]. While it has been known to all that cerebral white matter is sensitive to cerebral ischemic diseases[1]. In the context of cerebral ischemia and hypoxia, vitamin D deficiency makes it more difficult for maturation of oligodendrocyte and thus inducing white matter lesions, which are eventually displayed as WMH on MRI scans. Second, vitamin D is associated with vascular health. Some studies demonstrated that vitamin D could regulate the contractility of vascular smooth cells, maintain the stability of endothelial function[37] and depress the over-activation of renin–angiotensin–aldosterone system[38]. Third, vitamin D is known to own antioxidant and anti-inflammatory properties, as well as exerts a beneficial effect on immune system. Supplementing vitamin D could alleviate the burden of vascular damage associated with oxidative stress and modify the immune response during stroke[39-41], thereby improving the outcome of stroke[42]. Through these presumed mechanisms, 25(OH)D might contribute to the WMH of NICE patients.

Our results also showed that vitamin D was inversely related to AES-C score. Apathy is a behavioral syndrome characterized by a loss of motivation and occurs about as frequently as cognitive impairment and depression after stroke[43,44]. Despite of this, much less attention has been paid to apathy. To the best of our knowledge, this is the first report demonstrating an association between vitamin D and apathy. This prompts us to consider adopting vitamin D supplementation as an economical and safe option to improve the prognosis of NICE patients with apathy. Further prospective clinical trials are needed to provide a reliable conclusion.

The main limitation of the study is the cross-sectional design, which limits us to reveal a causal association between vitamin D with WMH and cognitive impairment. In addition, serum 25(OH)D were tested only at admission and the vitamin D status may be due to a consequence of limited sun exposure caused by physical impairment. Thirdly, the WMH was only evaluated by the Fazekas scale and not all characteristics were considered. Therefore, longitudinal studies were being carried out to confirm our present results and take more confounders into consideration.

Conclusion

In conclusion, our findings demonstrated that serum 25(OH)D levels were independently and significantly associated with WMH and cognitive impairment in patients with NICE. Future clinical trials should be conducted to explore the effect of vitamin D supplementation on WMH and changes of cognitive function in NICE patients.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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