ASSOCIATION OF SERUM 25(OH) D LEVELS WITH INFARCT VOLUMES AND STROKE SEVERITY IN ACUTE ISCHEMIC STROKE

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Abstract: *Background:* The aim of this study is to investigate whether 25-hydroxyvitamin D [25(OH) D] is associated with initial stroke severity and infarct volume, using diffusion-weighted imaging (DWI) in patients with acute ischemic stroke. *Methods:* We studied a total of 235 patients who were admitted within 24 hours of acute ischemic stroke onset. Initial stroke severity was assessed using the NIH Stroke Scale (NIHSS) score. Infarct volume was measured using DWI. Multivariable linear and logistic regression analyses were used to test whether 25(OH) D represents an independent predictor of infarct volume and stroke severity (NIHSS score of ≥6). *Results:* Among 235 study patients, the median age was 64 years (IQR 56-75 years), and 125 (53.2%) were women. In multivariable models adjusted for other significant risk factors, 25(OH) D levels in the lowest and second interquartiles were associated with an increased risk of a NIHSS≥6 (with highest 25 (OH) D quartile as reference) with odd ratios (OR) 3.02(95% confidence interval [CI]:1.59-6.34) and 5.85(2.90-11.54). The median DWI infarct volumes for the serum 25(OH) D level quartiles (lowest to highest) were 12.35, 6.55, 2.44, and 1.59 ml. The median DWI infarct volume in the lowest serum 25(OH) D level quartile was larger than that in the other 3 quartiles (P<0.001). The median adjusted DWI infarct volume in the lowest serum 25(OH) D level quartile was *statistically significantly larger than that in the other 3 quartiles (P<0.01). Conclusion:* In conclusion, reduced serum 25(OH) D levels in acute ischemic stroke are an early predictor of larger volumes of ischemic tissue and worse neurological deficit (assessed by the NIHSS).

Key words: 25-hydroxyvitamin D, neurological deficit, infarct volume, ischemic stroke, diffusion-weighted imaging.

Introduction

In 2013, stroke fell from the fourth to the fifth leading cause of death in the United States. Yet each year, ≈795 000 people continue to experience a new or recurrent stroke (ischemic or hemorrhagic) (1). Interestingly, ischemic stroke was more frequent and its proportion was higher than hemorrhagic stroke in Chinese populations (62.4% vs.27.5%) (2). Measurable biomarkers to predict illness development, severity and outcomes are pivotal for optimized care and allocation of healthcare resources (3).

Several previous studies have suggested a possible relationship between vitamin D and cardiovascular risk (4, 5) and cerebrovascular disease (6, 7). Specifically, a lot of evidence from epidemiological studies indicates that low serum 25-hydroxyvitamin D [25(OH) D] is associated with increased risk of strokes (8, 9) and worse functional outcomes (10). Majumdar et al. (11) suggested that regular monitoring of serum 25(OH) D levels and treatment of severe vitamin D deficiency, particularly in hypertensive subjects, could help in effective prevention of stroke.

Infarct volume is strongly correlated with clinical stroke severity and is therefore an important surrogate of stroke outcome (12). Turetsky et al. (13) found that low 25(OH) D was independently associated with larger ischemic infarct volume, which may partially explain observed worse outcomes in ischemic stroke patients with poor vitamin D status. Further, Dauba et al. (14) found that a low serum 25(OH) D level was a predictor of stroke severity at admission. However, the relationship between vitamin D and actual volumetric measurements of infarct size by diffusion-weighted imaging (DWI) has not been assessed previously. Thus, we propose a hypothesis that the concentration of serum 25(OH) D could be associated with stroke severity and infarct volume in patients with stroke. In the present study, we therefore investigated the effects of vitamin D on clinical stroke severity and infarct volume using DWI in patients who suffered from first-ever acute ischemic stroke (AIS).

Patients and methods

We sequentially screened patients with first-ever AIS who were admitted to Second Affiliated Hospital of Zhejiang University between October 2015 and September 2016. Patients were eligible for the study if they were admitted within 24 hours of stroke onset and exhibited evidence of acute ischemic stroke on DWI. The enrolled patients were newly diagnosed by a team consisting of two neurologists (Wang and Li), and had not received calcium and/or vitamin D therapy in the past 3 months. Patients with malignant tumor, survival time less than 24h, renal insufficiency (creatinine >1.5mg/dl), and autoimmune diseases were excluded. The present study has

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been approved by the ethics committee of the Second Affiliated Hospital of Zhejiang University. All participants or their relatives were informed of the study protocol and their written informed consents were obtained. Twenty-five out of the 235 included patients were incommunicable, and those consents were obtained from their relatives.

Demographic and clinical data were prospectively collected on admission, including information on patients' demographics (age, sex, body mass index [BMI]), prior and acute treatments, and history of risk factors (Table 1). Prior medications were determined by interviews with patients or their relatives, or preadmission prescriptions. Clinical severity was assessed at admission using the National Institutes of Health Stroke Scale (NIHSS). Strokes were classified according to the criteria of the TOAST (Trial of Org 10172 in Acute Stroke Treatment) classification (15). The clinical stroke syndrome was determined applying the criteria of the Oxfordshire Community Stroke Project (OCSP) (16). All blood samples were collected on the first day of admission under fasting state, and 25(OH) D was measured in accordance with standard detection methods by the E601 modular (Roche Diagnostics, Mannheim, Germany) with a calibration range from 3 to 70 ng/ml. The intra-assay coefficient of variation [CV] and inter-assay CV were 2.0-2.8%, 2.4%-3.4%, respectively. Other laboratory data, including values for high-sensitivity C-reactive protein (Hs-CRP), fasting blood glucose (FBG), total cholesterol, and low-density lipoprotein cholesterol were also tested.

Patients underwent imaging before receiving any reperfusion therapy using a 3.0-T scanner (Siemens Vision; Siemens Medical Systems, Erlangen, Germany) within 24 hours of hospitalization. Infarct volumes indicated by DWI were measured with MIPAV software (Medical Image Processing, Analysis, and Visualization, version 3.0; NIH, Bethesda, MD) (17). Acute diffusion lesions were identified on a slice-by-slice basis using a semiautomatic segmentation method, consulting apparent diffusion coefficients to distinguish acute from nonacute diffusion signals. DWI infarct volumes were calculated by multiplying slice thickness by total areas of lesions. The images were independently analyzed by 2 blinded and experienced stroke neurologists (Wang and Li). To assess inter-rater reliability, infarct volumes were measured by those two raters on a randomly selected subset of 20 patients, with greater than 0.90 set as the threshold for good agreement.

Statistical analysis

The results were expressed as percentages for categorical variables and as medians (interquartile ranges, IQRs) for continuous variables. The Mann-Whitney U test and chi-square test were used to compare the two groups. Spearman's Rank correlation was used for bivariate correlations. The influence of 25(OH) D levels on stroke severity (Moderate-to-high Stroke is defined as NIHSS score≥6) was performed by binary logistic regression analysis, which allows adjustment for possible confounding factors. For a more detailed exploration of the 25 (OH) D-stroke severity relationships, we also used multivariate analysis models for 25 (OH) D quartiles (with highest 25 (OH) D quartile as reference). Furthermore, the relationship between median DWI infarct volume and serum 25(OH) D level quartiles was evaluated using a semiparametric approach with univariate and multivariate quartile regression analysis (18). For the multivariate models, the median DWI infarct volumes were corrected for potential confounding variables. Categorical variables (sex, season of samples included, stroke subtype, stroke syndrome, vascular risk factors, and prior or acute treatment) and continuous variables (age, BMI, temperature, time from onset to blood collection, time to MR imaging, NIHSS score and serum levels of Hs-CRP and FBG) were used as covariates based on prior literature[17]. Results were expressed as adjusted odds ratios (OR) with the corresponding 95% Confidence interval (CI). All statistical analysis was performed with SPSS for Windows, version 20.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was defined as $P < 0.05$.

Results

Descriptive Characteristics of Stroke Patients

Two hundred thirty-five of 316 patients met the study criteria. Reasons for exclusion were initial examination outside of 24 hours (34 patients), unavailability of serum 25 (OH) D levels or MRI (41 patients) and died within 24 hours (6 patients). The median time between the stroke onset and the MRI was 7.5 hours (IQR, 3.6-15.4 hours), and the median DWI infarct volume was 4.33 ml (IQR 0.68–15.65). The baseline characteristics of the 235 patients presenting with acute ischemic stroke are described in Table 1.

25(OH) D levels were obtained at a median of 12.0 hours (IQR, 5.0-18.5 hours) after the stroke onset with a median value of 19.1ng/ml (IQR, 11.9–25.6ng/ml). Significant seasonal differences in 25(OH) D levels were observed (analysis of variance [ANOVA]: P=0.002), and 25(OH) D levels were highest during the summer and lowest during the winter. Factors associated with low vitamin D status were older age (P<0.001), atrial fibrillation (P=0.015), tobacco abuse (P=0.021) and cardioembolic stroke (P=0.009). There were no other differences in baseline clinical characteristics between serum 25(OH) D levels (P>0.05).

25(OH) D and stroke severity

There was a significantly negative correlation between levels of 25(OH) D and NHISS (r=-0.601; P<0.001). Patients in the lowest serum 25(OH) D level quartile had a higher median National Institutes of Health Stroke Scale score at admission compared with patients in the highest quartiles (14(IQR:10-19) VS. 3(2-5); P<0.001).

Table 1
Baseline characteristics of patients with stroke

	N=235
Age, medians (IQRs), years	64(56-75)
Female, (%)	125(53.2)
BMI, medians (IQRs), kg/m2	25.9(23.9-27.3)
NIHSS score at admission, median (IQR)	6(3-12)
Lesion volumes, median(IQR), ml	4.33(0.68-15.65)
Time to MRI, median (IQR), h	7.5(3.6-15.4)
Time to blood collected, median (IQR), h	12.0(5.0-18.5)
Winter included, no. (%)	73(26.4)
Prior vascular risk factors, no. (%)	
Hypertension	161(68.5)
Diabetes	62(26.4)
Coronary artery disease	53(22.6)
Atrial fibrillation	39(16.6)
Current smoking	32(13.6)
PVD	21(8.9)
Pre-stroke treatment, no. (%)	
Anti-platelet agents	121(51.5)
Statins	66(28.1)
Acute treatment, no. (%)	
IV thrombolysis	36(15.3)
Mechanical thrombectomy	25(10.6)
IV thrombolysis and/or mechanical thrombectomy	53(22.6)
Stroke etiology no. (%)	
Cardioembolic	49(20.9)
Small-vessel occlusive	70(29.8)
Large-vessel occlusive	72 (30.6)
Other	24(10.2)
Unknown	20(8.5)
Hs-CRP, mg/dL	0.48(0.32-0.96)
FBG, mmol/L	5.65(4.99-6.22)
25(OH) D, ng/ml	19.1(11.9-25.6)

IQR, interquartile range; BMI, body mass index; NIHSS, National Institutes of Health Stroke Scale; PVD, peripheralvascular disease; Hs-CRP: high-sensitivity C-reactive protein; FBG: fasting blood glucose; MRI: Magnetic Resonance Imaging

At admission, 112 patients (47.7%) had a minor stroke (NIHSS≤5). In these patients, the mean 25 (OH) D level was higher than that observed in patients with moderate-to-high clinical severity [24.5(IQR: 20.1-29.3)ng/ml VS. 12.8(8.5-18.4)ng/ml; P<0.001; Fig 1). In univariate logistic regression analysis, we calculated the ORs of 25 (OH) D levels as compared with other risk factors. With an unadjusted OR of 0.808 (95% CI, 0.767-0.852; P<0.001), 25 (OH) D had a strong association with moderate-to-high clinical severity stroke. After adjusting for all other significant indictors in univariate logistic regression analysis, 25 (OH) D remained an independent stroke severity predictor with an adjusted OR of 0.886 (95% CI, 0.805 –0.932). For a more detailed exploration of the 25 (OH) D-stroke severity relationships, we also used multivariate analysis models to estimate adjusted OR and 95% CIs of moderate-to-high stroke for 25 (OH) D quartiles (with highest 25 (OH) D quartile as reference). In multivariable models adjusted for age, gender, and other significant risk factors, 25(OH) D levels in the lowest and second interquartiles were associated with an increased risk of a NIHSS≥6 (Table 2).

Figure 1
Serum levels of 25(OH) D in stroke patients with minor and moderate-to-high clinical severity. Mann–Whitney U-test. All data are medians and interquartile ranges (IQR). Moderate-to-high Stroke is defined as NIHSS score≥6. NIHSS=National Institutes of Health Stroke Scale

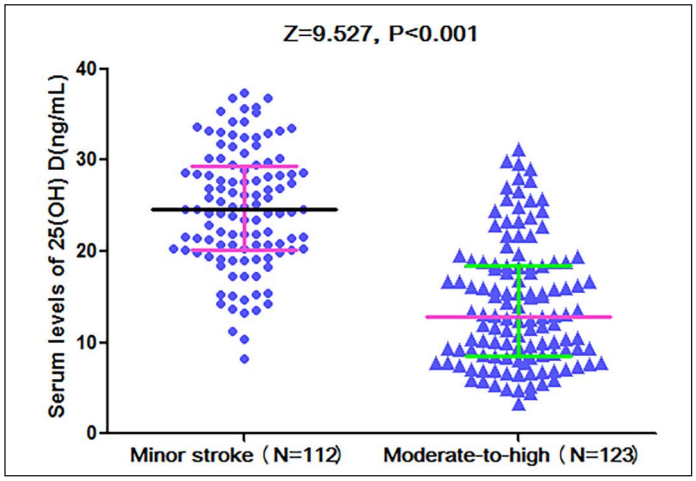


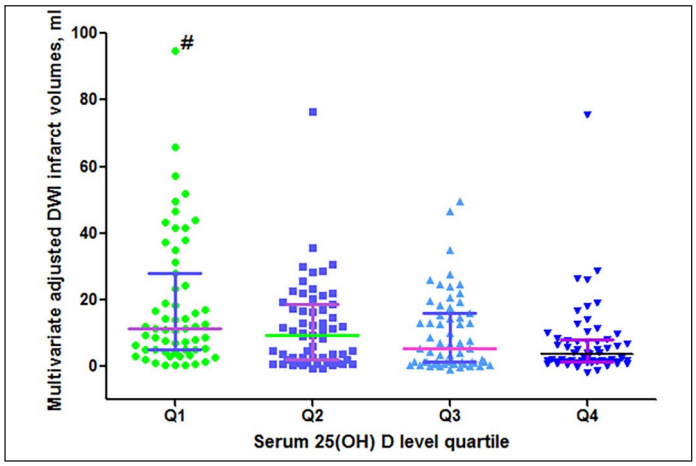
Table 2
Multiple Stepwise Logistic Regression Analysis Showing Factors Independently Associated with the Moderate-to-High Stroke at Baseline*

Parameters **	OR	95%CI	P
Q1 VS Q4	5.85	2.90-11.54	<0.001
Q2 VS Q4	3.02	1.59-6.34	0.002
Q3 VS Q4	1.49	0.98-2.98	0.075
Q1-3 VS Q4	3.15	1.50-6.68	0.001

* Variables included in the original model are age, sex, BMI, infarct volume, season of samples included, time from onset to blood collection and MRI, stroke syndrome, stroke etiology, vascular risk factors, acute treatment, serum levels of Hs-CRP, FBG and 25(OH) D in Quartiles. Moderate-to-high Stroke is defined as NIHSS score≥6. ** Serum 25(OH) D levels in Quartile 1 (<11.9ng/ml), Quartile 2 (11.9–19.1 ng/ml), Quartile 3 (19.2–25.6 ng/ml), and Quartile 4 (>25.6ng/ml); Q4 as reference with OR=1. BMI, body mass index; Hs-CRP: high-sensitivity C-reactive protein; FBG: fasting blood glucose; NIHSS, National Institutes of Health Stroke Scale

Figure 2

Scatter plots showing multivariate adjusted median diffusion-weighted magnetic resonance imaging (DWI) infarct volumes (Intermediate line), interquartile range (up and bottom lines), and minimum and maximum values across the range of serum 25(OH) D level quartiles



#Diffusion-weighted magnetic resonance imaging volume of quartile 1 greater than that of quartiles 2, 3, and 4 (P<0.01). Serum 25(OH) D levels in Quartile 1 (<11.9ng/ml), Quartile 2 (11.9–19.1 ng/ml), Quartile 3 (19.2–25.6 ng/ml), and Quartile 4 (>25.6ng/ml)

25(OH) D and infarct volume

Patients in the lowest serum 25(OH) D level quartile had a higher median infarct volume at admission compared with patients in the highest quartiles (12.35(IQR: 5.29-50.59) ml VS. 1.59(0.62-4.65) ml; P<0.001). The median DWI infarct volumes for the serum 25(OH) D level quartiles (lowest to highest) were 12.35, 6.55, 2.44, and 1.59 ml (Table 3). The distribution of infarct volumes was positively skewed, reflecting a larger proportion of patients with smaller infarct volumes (skewness, 2.9; kurtosis, 11.8). Nonparametric Spearman rank correlation revealed a statistically significant negative correlation between serum 25(OH) D level and infarct volume (r=–0.417; P<0.001).

The results of the univariate and multivariate analyses of the relationship between serum 25 (OH) D levels at admission and infarct volume are given in Table 3. Univariate analysis indicated that the overall quartiles regression model for the relationship between median infarct volume and serum 25(OH) D quartiles was statistically significant, with a negative slope (P<0.001). The median infarct volume in the lowest serum 25(OH) D level quartile was statistically significantly larger than those in the upper 3 quartiles (P<0.05). The multivariate analysis indicated again a statistically significant overall trend toward increased DWI infarct volumes across 25(OH) D quartiles (P<.001). Interestingly, the median adjusted DWI infarct volumes and interquartile range for each of the serum 25(OH) D level quartiles are shown in the Figure 2 and were 11.3(IQR, 4.9-27.9); 9.2(2.0-18.5); 5.4(1.2-16.1) and 3.8(1.4-8.1) ml from lowest to highest. Similarly, the median adjusted DWI infarct volume in the lowest serum 25(OH) D level quartile was larger than that in the upper 3 quartiles (P<0.05).

Discussion

In this study, the findings show that serum levels of 25(OH) D at admission are inversely associated with the stroke severity and volume of DWI abnormality. Patients with 25(OH) D in the lowest quartiles had DWI infarct volumes quadrupling as large as those in the upper 3 quartiles (12.35 vs. 2.55ml), and this difference persisted after adjusting for potential confounding factors. It also has been shown that a mismatch volume of more than 10 ml is predictive of lesion progression and early neurological deterioration (19). Yoo et al. (20) found that among patients with anterior circulation acute ischemic stroke who undergo intra-arterial therapy, final infarct volume is a critical determinant of 3-month functional outcome. Thus, the detection of infarct volume is used for monitoring and management of stroke patients. Combined with our data, it is imperative to emphasize targeted more frequent medical interventions for stroke patients, especially for these patients with the concentration of 25(OH) D in the lowest quartiles (<11.9ng/ml).

Those findings are consistent with previous studies that assessed serum levels of 25(OH) D in patients with ischemic

Table 3

Relationship between Serums 25(OH) D Level Quartile at Admission and DWI Infarct Volume Based on Univariate and Multivariate Quartile Regression Analysis

Serum 25(OH) D Quartile*	Median Infarct Volume (ml, IQR)**	Multivariate Change in Infarct Volume (95% CI)**,ξ	P value
Q1	12.35(5.29-50.59)	6.22(3.02-12.54)	<0.001
Q2	6.55(1.43-16.54)	3.36(1.55-6.92)	0.001
Q3	2.43(0.51-11.33)	1.42(0.90-2.55)	0.15
Q4	1.59(0.62-4.55)	1[Reference]	—
Q1-3	6.76(1.47-18.74)	3.40(1.53-7.02)	<0.001

* Serum 25(OH) D levels in Quartile 1 (<11.9ng/ml), Quartile 2 (11.9–19.1 ng/ml), Quartile 3 (19.2–25.6 ng/ml), and Quartile 4 (>25.6ng/ml); Q4 as reference with OR=1; ** P<0.001 for trend across quartiles; ξ Adjusted for age, sex, BMI, NIHSS Score, season of samples included, time from onset to blood collection and MR imaging , stroke syndrome, stroke etiology, vascular risk factors, acute treatment, serum levels of Hs-CRP, FBG and 25(OH) D in Quartiles; DWI: Diffusion-Weighted Magnetic Resonance Imaging; BMI, body mass index; Hs-CRP: high-sensitivity C-reactive protein; FBG: fasting blood glucose; NIHSS, National Institutes of Health Stroke Scale;

stroke. Tu et al. (21) found that serum 25(OH) D levels reduced with increasing severity of stroke as defined by the NIHSS score, and there was a negative correlation between levels of 25(OH) D and the NIHSS (P< 0.001). Furthermore, significantly inversely correlation between levels of 25(OH) D and the infarct volume (P<0.001) had been proposed in recent studies (22, 23). It has been suggested that vitamin D has neuroprotective properties (24), and vitamin D supplementation could contribute to reducing the volume of cerebral infarct in animal models of stroke (25). In a rat model of cerebral ischemia induced by ligation of the middle cerebral artery, pretreatment with 1, 25(OH) 2D for 8 days was associated with a significant reduction in infarct size of the ischemic brain (26).

Pathophysiological mechanisms remain speculative but several possible biologic mechanisms might explain the association of Low 25(OH) D with stroke severity and volume of DWI abnormality, involving both a dysregulation of the inflammatory response as well as suppression of known neuroprotectants such as insulin-like growth factor 1(IGF-1) (27). A study suggested that treatment with the antiinflammatory cytokine IFN-beta affords significant neuroprotection against ischemia/reperfusion injury in the rat (28). Further, Ma et al. (29) found that intranasal administration of TGF-β1 reduces infarct volume, improves functional recovery and enhances neurogenesis in mice after stroke. Second, it may reduce thrombus burden via reduction of plasminogen activator inhibitor and thrombospondin1 and secretion of tissue plasminogen activator (30). Lastly, vitamin D has been shown to mediate vasodilatation via potentiation of nitric oxide action (13), which has been demonstrated to improve penumbral blood flow and thus neuronal survival in ischemic stroke via reactive vasodilation in collateral vessels (31).

Several limitations must be acknowledged, including a relatively small population size (N=235) and a single center. Second, although our data show a significant association between 25(OH) D and infarct volume and stroke severity, the observational nature of study made it impossible to determine causality of the observed associations. Third, we adjusted for many risk factors in the multivariate analysis, but the possibility of residual confounding remains. We cannot exclude the possibility that residual confounding factors. Lastly, all blood samples were collected on the first day of admission under fasting state. Biochemical values were modified before and after onset of stroke. Thus, we cannot determine the values of 25(OH) D before stroke onset.

Conclusions

Reduced serum 25(OH) D levels in acute ischemic stroke are an early predictor of larger volumes of ischemic tissue and worse neurological deficit (assessed by the NIHSS). Further investigations need to elucidate the mechanism of this effect and to assess the role of vitamin D as a prognostic variable and of vitamin D modulation as part of a potential neuroprotective strategy.

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Disclosure statement: The authors report no declarations of interest.

Ethical Standards: The present study has been approved by the ethics committee of the Second Affiliated Hospital of Zhejiang University. All participants or their relatives were informed of the study protocol and their written informed consents were obtained.

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