LOW SERUM LEVELS OF 25-HYDROXYVITAMIN D ARE ASSOCIATED WITH STROKE RECURRENCE AND POOR FUNCTIONAL OUTCOMES IN PATIENTS WITH ISCHEMIC STROKE

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Abstract: *Objective:* To evaluate the association between serum 25(OH) D levels and functional outcome and stroke recurrence events in a 6-month follow-up study in a cohort of patients with an acute ischemic stroke (AIS). *Methods:* From March 2014 to August 2015, consecutive first-ever AIS patients admitted to the Department of Emergency of our hospital were identified. Serum 25(OH) D levels were measured at admission. Functional outcome was evaluated at 6-month using the modified Rankin scale (m-Rankin). We used logistic regression models to assess the relationship between 25(OH) levels and risk of recurrent stroke or functional outcome. *Results:* We recorded 277 stroke patients. There were significantly negative correlation between levels of 25(OH) D and NHISS (P<0.001), and the infarct volume (P< 0.001). Thirty-one patients (11.9%) had a stroke recurrence, while 82 patients (29.6) were with poor functional outcomes. In multivariate logistic regression analyses, serum 25(OH) D level was an independent marker of poor functional outcome and stroke recurrence [odds ratio (OR) 2.55 (1.38-3.96) and 3.03(1.65–4.12), respectively, P<0.001 for both, adjusted for NHISS, other predictors and vascular risk factors] in patients with AIS. *Conclusion:* Our results demonstrate that low 25(OH) D levels are associated with stroke recurrence and support the hypothesis that 25(OH) D may serve as a biomarker of poor functional outcome after stroke.

Key words: 25-hydroxyvitamin D, stroke recurrence, ischemic stroke, functional outcome.

Introduction

Stroke is the second leading cause of mortality and it causes a tremendous burden on health resources in China (1). Early and accurate prediction of outcome in stroke is important and influences risk-optimized therapeutic strategies. Thus, it is important to identify those stroke patients at high risk for stroke recurrence and outcome.

Vitamin D deficiency is highly prevalent due to lifestyle and environmental factors which limit sunlight induced vitamin D production in the skin (2). Literature documented that suboptimal intake of vitamin D can not only lead to bone loss, but also increase the risk of many common and serious diseases, including dementia(3), cancers, diabetes, cardiovascular diseases, as well as stroke (4). Previous studies have indicates that vitamin D deficiency is associated with increased risk of strokes (5, 6). A meta-analysis finished by Brøndum-Jacobsen et al. (7) found a stepwise increasing risk of symptomatic ischemic stroke with decreasing concentration of plasma 25-hydroxyvitaminD [25(OH) D]. The relationship between serum 25(OH) D and functional outcomes had been suggested in previous studies (2, 8-10). In this study, we aimed to evaluate the association between serum 25(OH) D levels and functional outcome and stroke recurrence events in a 6-monthfollow-up study in a cohort of patients with an acute ischemic stroke (AIS).

Methods

From March 2014 to August 2015, consecutive first-ever AIS patients admitted to the Department of Emergency of the first people's hospital of Shangqiu city, China, were identified. The study population was exclusively Chinese. The clinical diagnoses of ischemic stroke were validated on the basis of Magnetic Resonance Imaging (MRI). The enrolled patients were newly diagnosed by a team consisting of two neurologists (Ji and Zhou), and had not received calcium and/or vitamin D therapy in the past 12 months. Patients with malignant tumor, intracerebral hemorrhage, head trauma, severe edema and autoimmune diseases were also excluded. The present study has been approved by the ethics committee of the first people's hospital of Shangqiu city. All participants or their relatives were informed of the study protocol and their written informed consents were obtained.

Clinical information was collected. Demographic data (age and sex), body mass index (BMI), and history of risk factors (hypertension, diabetes mellitus, atrial fibrillation, hyperlipidemia, smoking habit and alcohol abuse) were obtained at admission. Pre-stroke therapy, including oral anticoagulants, antiplatelet agents, antihypertensive treatment, and statins, as well as acute treatment (IV thrombolysis and/or mechanical thrombectomy) was recorded. Clinical severity was assessed at both admission anddischarge using the National Institutes of Health Stroke Scale (NIHSS). Functional impairment was evaluated at 6-month using the modified Rankin scale (m-Rankin). Strokes were classified according to

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the criteria of the TOAST (Trial of Org 10172 in Acute Stroke Treatment) classification (11). The clinical stroke syndrome was determined applying the criteria of the Oxfordshire Community Stroke Project (OCSP) (12). MRI with diffusion-weighted imaging (DWI) was available. The infarct volume was calculated by using the formula 0.5×a×b×c (13).

Serum 25(OH) D levels were measured at baseline. 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 in the hospital biochemistry department of this hospital. Serum 25(OH) D levels were measured on 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 1.8-2.6%, 2.1%-3.0%, respectively.

We followed the participants for a median of 6 months using a standard questionnaire, and telephone or household contact by physician investigators. The primary end-point was functional outcome (assessed by mRS score) on 6-month after stroke onset. The secondary endpoint was stroke recurrence. Stroke recurrence was defined as sudden functional deterioration in neurological status with decrease of NIHSS score of 4 or more, or a new focal neurological deficit of vascular origin lasting >24 hours.

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 functional outcome and stroke recurrence were performed by binary logistic regression analysis, which allows adjustment for confounding factors (age, sex, BMI, stroke syndrome, stroke etiology, the NIHSS score, infarct volume, vascular risk factors, hospital stays and admission seasons). Results were expressed as adjusted odds ratios (OR) with the corresponding 95% Confidence interval (CI). Further, receiver operating characteristic curves (ROC) was used to test the overall accuracy of the 25(OH) D and other markers to predict stroke recurrence and functional outcome. 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

We recorded 277 stroke patients. The 25 (OH) D levels were obtained in those patients with a median value of 17.2ng/ml (IQR, 12.7–21.9ng/ml). Overall median age was 65 (IQR, 56-75) and 40.1% were female. The baseline characteristics of the 277 patients presenting with acute ischemic stroke are described in Table 1.

Significant seasonal differences in 25(OH) D levels were observed (analysis of variance [ANOVA]: P=0.004), and 25(OH) D levels were highest during the summer and lowest during the winter (18.9[IQR, 13.4-23.6] ng/ml and 15.1[10.3-18.2]ng/ml, respectively). In addition, 25(OH) D levels were with no differences between sexes, ages of patients, gender, risk factors, stroke subtype distribution, stroke etiology and pre-stroke treatment (P>0.05, respectively). Interestingly, there were significantly negative correlation between levels of 25(OH) D and NHISS (P<0.001), and the infarct volume (P< 0.001). Further, there was still a significant negative trend between serum 25(OH) D levels and NIHSS score (P=0.021), using ordered logistic regression after multivariate adjustment for possible confounders: age, hospital stay, seasons, risk factors, stroke subtype and syndrome. In addition, there was a significantly negative correlation between levels of 25(OH) D and BMI (r=-0.399, P<0.001).

Table 1
Baseline characteristics of patients with stroke

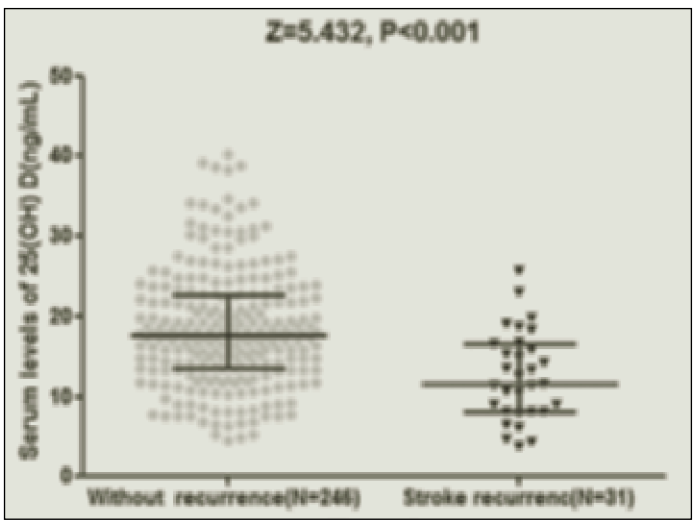
	N=277
Age, years medians (IQRs)	65(56-75)
Female, (%)	111(40.1)
BMI, kg/m2 medians (IQRs)	25.9(23.7-27.4)
Winter included, no. (%)	73(26.4)
Prior vascular risk factors, no. (%)	
Hypertension	165(59.6)
Diabetes	78(28.2)
Hypercholesterolemia	81(29.2)
Smoking	73(26.4)
Atrial fibrillation	62(22.4)
Myocardial infarction	34(12.3)
Previous TIA	37(13.4)
PVD	15(5.4)
Pre-stroke treatment, no. (%)	
Anti-platelet agents	85(30.7)
Anti-hypertensive treatment	143(51.6)
Anticoagulants	21(7.6)
Statins	65(23.5)
Acute treatment, no. (%)	
IV thrombolysis	49(17.7)
Mechanical thrombectomy	22(7.9)
IV thrombolysis and/or mechanical thrombectomy	66(23.8)
NIHSS at admission, medians (IQR)	8(4-12)
Lesion volumes, ml median(IQR)	26(8-42)
Rankin at 6-month, median(IQR)	1(0-3)
Stroke etiology no. (%)	
Small-vessel occlusive	71(25.6)
Large-vessel occlusive	66(23.8)
Cardioembolic	102(36.8)
Other	25(9.0)
Unknown	13(4.7)

IQR, interquartile range; NIHSS, National Institutes of Health Stroke Scale; PVD, peripheralvascular disease; TIA, transient ischemic attack; BMI, body mass index

In our study, thirty-one patients (11.2%) had a stroke recurrence. Serum 25(OH) D levels in patients with recurrent stroke were significant lower as compared with those in patients without recurrent stroke [11.2(IQR, 8.6-16.5)ng/Ml vs. 17.6(IQR, 13.5-22.7) ng/Ml; Z=5.432, P<0.001; Fig 1]. In multivariate analyses, a 25(OH) D level in the lowest interquartile (<12.7ng/ml) was associated with a higher risk of stroke recurrence (OR = 3.03; 95% CI = 1.65–4.12; P<0.001) (Table 2). In addition, based on the ROC curve, the optimal cutoff value of serum 25[OH] D levels as an indicator for screening of stroke recurrence was estimated to be 11.7ng/ml, which yielded a sensitivity of 82.3 % and a specificity of 64.5%, Fig 2A. With an AUC of 0.82 (95% CI, 0.76–0.89), 25 (OH) D showed a significantly greater discriminatory ability to predict stroke recurrence as compared with Hs-CRP (AUC, 0.63; 95% CI, 0.57–0.72; P<0.001), HCY (AUC, 0.67; 95% CI, 0.60–0.73; P<0.001), and NIHSS score (AUC, 0.60; 95% CI, 0.54–0.66; P<0.001).

Figure 1

Distribution of serum levels of 25(OH) D in stroke patients with stroke recurrence and without stroke recurrence. Horizontal lines represent medians and inter-quartile ranges (IQR). P values refer to Mann-Whitney U tests for differences between groups



At 6-month follow-up, a poor functional outcome was found in 82 patients (29.6%) with a median mRS score of 4 (IQR, 3–6). Serum 25(OH) D levels in patients with a good outcome were significantly greater than those in patients with a poor outcome (18.3 [IQR, 14.2–23.1] vs. 14.3 [IQR, 9.1–17.8] ng/Ml; Z=4.418; P<0.0001; Figure 3.). In multivariate analyses, a 25(OH) D level in the lowest inter-quartile (<12.7ng/ml) was associated with a higher risk of poor functional outcome (OR=2.55; 95% CI =1.38–3.96; P=0.001) (Table 2). In addition, based on the ROC curve, the optimal cutoff value of serum 25[OH] D levels as an indicator for screening of poor functional outcome was estimated to be 14.5ng/ml, which yielded a sensitivity of 71.7 % and a specificity of 62.3%, Fig 2B. With an AUC of 0.76 (95% CI, 0.70–0.82), 25 (OH) D showed a significantly greater discriminatory ability to predict stroke recurrence as compared with Hs-CRP (AUC, 0.62; 95% CI, 0.55–0.70; P<0.001), HCY (AUC, 0.65; 95% CI, 0.59–0.72; P<0.01), and NIHSS score (AUC, 0.66; 95% CI, 0.60–0.72; P<0.01).

Figure 2

Receiver operating characteristic curves (ROC) was used to test the overall accuracy of the 25(OH) D to predict stroke recurrence and poor functional outcome. (A) ROC curve was utilized to evaluate the accuracy of serum 25(OH) D levels to predict stroke recurrence; (B) ROC curve was utilized to evaluate the accuracy of serum 25(OH) D levels to predict poor functional outcome

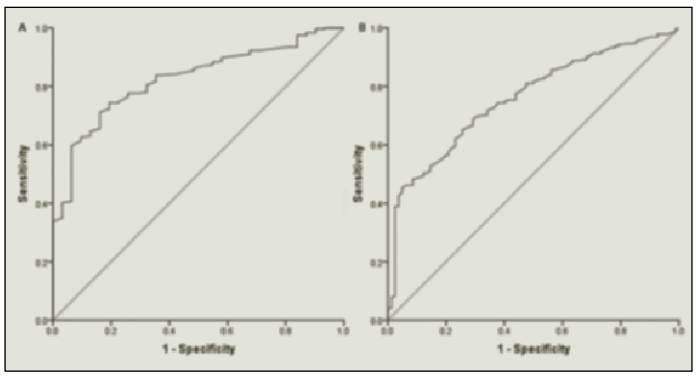


Figure 3

Distribution of serum levels of 25(OH) D in stroke patients with poor functional outcomes and good functional outcomes. Horizontal lines represent medians and inter-quartile ranges (IQR). P values refer to Mann-Whitney U tests for differences between groups

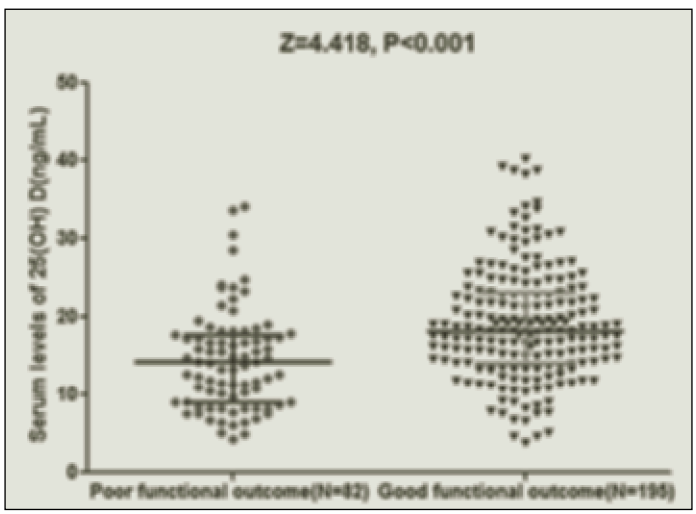


Table 2
Multivariate analysis of predictors of stroke recurrence and poor functional outcomes [a]

Predictors	OR	95% CI	P
Stroke recurrence			
25(OH)D <12.7ng/ml [b]	3.03	1.65-4.12	<0.001
NIHSS (per unit increase)	1.21	1.06-1.35	0.005
Infarct volume(per unit increase)	1.26	1.12-1.78	0.01
Stroke etiology (Vessel occlusive)[c]	4.12	2.47-8.15	0.009
Anticoagulants(yes vs. no)	0.92	0.83-0.97	0.03
IV thrombolysis and/or mechanical thrombectomy	0.33	0.21-0.49	<0.001
Poor functional outcomes			
25(OH)D <12.7ng/ml [b]	2.55	1.38-3.96	0.001
NIHSS (per unit increase)	1.31	1.16-1.54	0.007
Infarct volume(per unit increase)	1.21	1.10-1.45	0.003
Anticoagulants(yes vs. no)	3.93	1.26-10.32	0.02
IV thrombolysis and/or mechanical thrombectomy	0.28	0.10-0.55	<0.001
Stroke etiology (Cardioembolic)	3.15	2.04-5.32	0.006

a. Multivariable model included all of the following variables: age, gender, BMI, infarct volume, NIHSS score, season of samples included, time from onset to blood collection, stroke syndrome, stroke etiology, vascular risk factors and serum levels of Hs-CRP and HCY; b .25(OH)D>12.7 ng/ml corresponding to the combination of the upper 3 IQR used as the reference; c. Other ischemic stroke subtype as the reference; OR, odds ratio; CI, confidence interval; NIHSS, National Institutes of Health Stroke Scale

Discussion

This study demonstrated that low 25(OH) D levels were independently associated with both the stroke recurrence events and a poor functional prognosis in stroke patients. The association is dose dependent and independent of other traditional cerebrovascular risk factors. We also found that the serum 25(OH) D levels at admission had an inversely correlated with infarct volume and the admission neurological deficit (assessed by the NIHSS).

Several limitations must be acknowledged, including a relatively small population size and recurrence events (N=30). Second, our data came from a hospital-based registry, which could have hospital selection bias. In addition, some patients who had died also suffered from recurrent stroke. Thus, our conclusion may be underestimated. Third, the observational nature of study made it impossible to determine causality of the observed associations. Fourth, Schneider et al. (14) reported that persons with low 25(OH) D who are genetically predisposed to high vitamin D binding protein (DBP; rs7041 G, rs4588 A alleles), who therefore have lower predicted bioavailable 25(OH) D, may be at greater risk for stroke. However, we did not obtain the information about DBP. Lastly, 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.

In our study, 11.2% of the stoke patients had a stroke recurrence in the 6-month follow-up. Similarly, Coutts et al. (15) found that after a transient ischemic attacks or minor stroke, there was an estimated 10% risk of recurrent stroke within 90 days .The overall rate of recurrence after stroke in previous studies was in the range from 3.8% to 17.7% (16-20). The differences might have been caused by different settings of the studies, different follow-up time, different diagnostic methods, and different ethnicities studied. In addition, Oliai et al. (21) reported that higher BMI and higher body fat percentage were significantly associated with lower serum 25(OH) D levels in older persons. Consistent with this result, we found that there was a significantly negative correlation between levels of 25(OH) D and BMI (P<0.001).

The reasons why a low 25(OH) D level may increase the risk of stroke recurrence and poor functional prognosis remain uncertain. It could be hypothesized that it may result from an increase in vascular events in patients with 25 (OH) D deficiencies (22). Second, hyperparathyroidism has been proposed as one mechanism by which vitamin D deficiency could mediate cardiovascular events, since it may promote cardiac hypertrophy, vascular remodeling and inflammation (23). Third, animal studies have shown that vitamin D receptors (VDRs) and vitamin D are key molecules to brain development and VDRs knockout mice have muscular and motor impairment (24). Lastly, some data indicate that vitamin D deficiency may contribute to systemic inflammation (25). Reduced 25(OH) D might be associated with overall increased inflammatory activity (26).

Conclusion

To conclude, our results demonstrate that low 25(OH) D levels are associated with stroke recurrence and support the hypothesis that 25(OH)D may serve as a biomarker of poor function outcome after stroke. Further investigations are needed to determine whether vitamin D supplementation could be of interest in the acute stage of stroke.

Compliance with Ethical Standards: Disclosure of potential conflicts of interest: On behalf of all authors: Weidong Ji, Haiyun Zhou, Suishan Wang, Li Cheng, Yan Fang, the corresponding author (Yan Fang) states that there is no conflict of interest. *Research involving human participants:* This study was approved by the Institutional Review Board of the first people's hospital of Shangqiu city. *Informed consent:* Informed written consent was obtained from each patient, family, or legal guardian.

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