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ORIGINAL PAPER

Prognostic Value of Serum 25-Hydroxyvitamin D in Patients with Stroke

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Abstract We aimed to evaluate the association between 25-hydroxyvitamin D [25(OH) D] levels and both clinical severity at admission and outcome at discharge in patients with acute ischemic stroke (AIS). From June 2012 to October 2013, consecutive first-ever AIS patients admitted to the Department of Emergency of The Fourth Affiliated Hospital of Harbin Medical University, China were identified. Clinical information was collected. Serum 25(OH) D levels were measured at baseline. Stroke severity was assessed at admission using the National Institutes of Health Stroke Scale (NIHSS) score. Functional outcome was evaluated at discharge using the modified Rankin scale (m-Rankin). Multivariate analyses were performed using logistic regression models. During the study period, 326 patients were diagnosed as AIS and were included in the analysis. Serum 25(OH) D levels reduced with increasing severity of stroke as defined by the NIHSS score. There was a negative correlation between levels of 25(OH) D and the NIHSS ($r = -0.389$, $P = 0.000$). In multivariate analyses, serum 25(OH) D level was an independent prognostic marker of discharge favorable functional outcome and survival [odds ratio 3.96 (2.85–7.87) and 3.36 (2.12–7.08), respectively, $P = 0.000$ for both, adjusted for NHISS, other predictors and vascular risk factors] in patients with AIS. Serum 25(OH) D levels are a predictor of both severity at admission and favorable functional outcome in patients with AIS. Additional research is needed on vitamin D supplementation to improve the outcome of post-stroke patients.

Keywords 25-Hydroxyvitamin D · Acute ischemic stroke · Prognosis

Introduction

Stroke is the second leading cause of mortality and it causes a tremendous burden on health resources in China [1]. Interestingly, vitamin D deficiency has been reported to contribute to the risk of cardiovascular disease, especially stroke [2–4]. Witham et al. [3] found that high dose oral vitamin D supplementation produced short-term improvement in endothelial function in stroke patients with well-controlled baseline blood pressure.

Several mechanisms may explain the link between vitamin D deficiency and stroke. First, the present data highlight a potential interaction between vitamin D deficiency and hypertension [5]. Second, vascular smooth muscle cells and endothelial cells express receptors for vitamin D. Putative vascular effects of vitamin D are wide-ranging and include modulation of smooth muscle cell proliferation [6], inflammation [7], and thrombosis [8]. Third, vitamin D deficiency triggers secondary hyperparathyroidism. Parathyroid hormone promotes myocyte hypertroph, vascular remodeling, and has a proinflammatory effect [5].

Many forms of vitamin D can be measured in the blood, and given its long half-life, 25-hydroxyvitamin D [25(OH) D] appears to be the appropriate biomarker to determine

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total body vitamin D stores, even though it is not the active metabolite that binds to the vitamin D receptor [9]. Previous studies have reported that deficient 25(OH) D levels are associated with cardiovascular disease (CVD) events and mortality [10]. One study observed stepwise increasing risk of symptomatic ischemic stroke with decreasing plasma 25(OH) D concentrations [11]. Daubail et al. [12] reported that a low serum 25(OH) D level is a predictor of both severity at admission and poor early functional outcome in stroke patients. We aimed to evaluate the association between serum 25(OH) D levels and functional outcome at discharge in a cohort of Chinese patients with an acute ischemic stroke (AIS).

Subjects and Methods

From June 2012 to October 2013, consecutive first-ever AIS patients admitted to the Department of Emergency of The Fourth Affiliated Hospital of Harbin Medical University, China were identified. All patients were admitted within 24 h of experiencing a new focal or global neurological event. Brain imaging (either CT or MRI) was performed routinely within 24 h after admission. The enrolled patients with AIS were newly diagnosed by a team consisting of two neurologists, 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 Institutional Review Committee on Human Research of our hospital approved the study protocol. All patients received oral and written information concerning the background and procedures of the study, and the patients or their relatives gave written informed consent prior to entering the study.

Clinical information was collected. Demographic data (age and sex) and history of risk factors (hypertension, diabetes mellitus, atrial fibrillation, hyperlipidemia, smoking habit and alcohol abuse) were obtained at admission. Stroke subtype was classified according to TOAST (Trial of Org 10172 in Acute Stroke Treatment) criteria [13], which distinguished large-artery arteriosclerosis, small-artery occlusion, cardioembolism, other causative factor, and undetermined causative factor. The clinical stroke syndrome was determined by applying the criteria of the Oxfordshire Community Stroke Project: total anterior circulation syndrome; partial anterior circulation syndrome; lacunar syndrome; and posterior circulation syndrome [14].

The National Institutes of Health Stroke Scale (NIHSS) score (scores range from 0 to 42, with greater scores indicating increasing severity) was assessed on admission [15]. Functional outcome was obtained at discharge according to the modified Rankin Scale (mRS) score [16]. The primary end point of this study was favorable functional outcome of stroke patients after discharge from baseline (mRS, 0–2). Secondary end point in stroke patients was death from any cause in hospital. Outcome assessment was performed by one trained medical student with a structured follow-up telephone interview with the patient or, if not possible, with the closest relative.

Brain imaging (either CT or MRI) was performed routinely within 24 h after admission. MRI with diffusion-weighted imaging (DWI) was available in some patients. In those patients, DWI lesion volumes were determined by one experienced neurologist unaware of the clinical and laboratory results. The infarct volume was calculated by using the formula $0.5 \times a \times b \times c$ (where a is the maximal longitudinal diameter, b is the maximal transverse diameter perpendicular to a and c is the number of 10-mm slices containing infarct) [17].

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 25(OH) D levels were therefore used to classify the vitamin D status into vitamin D deficiency (<20 ng/ml) and vitamin D insufficiency (20–30 ng/ml) [18].

Results are expressed as percentages for categorical variables and as medians (interquartile ranges, IQRs) for the continuous variables. Univariate data on demographic and clinical features were compared by Mann–Whitney U-test or Chi Square test as appropriate. Correlations among continuous variables were assessed by the Spearman rank-correlation coefficient. The influence of 25(OH) D levels on functional outcome was performed by binary logistic regression analysis, which allows adjustment for confounding factors (age, sex, 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). In logistic regression analysis, we calculated the ORs of log-transformed 25(OH) D levels as compared with the NIHSS score and other risk factors. 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

Baseline Characteristics of Study Samples

During the study period, 326 patients were diagnosed as AIS and were included in the analysis. The median age was

Table 1 Basal characteristic of patients with acute ischemic stroke

Baseline characteristics	Total (n = 326)	Serum 25(OH) D levels		P^a
		<20 ng/ml (n = 223)	≥20 ng/ml (n = 103)	
Median age, yr (IQR)	65 (57–75)	69 (61–83)	61 (52–70)	<0.001
Female sex (%)	39.6	39.5	39.8	NS
Hypertension (%)	72.1	71.3	73.8	NS
Time to inclusion, hours (IQR)	6.8 (3.5–11.3)	6.7 (3.5–11.6)	6.8 (3.4–11.2)	NS
Hospital stay, days (IQR)	31 (15–48)	38 (21–59)	26 (12–40)	0.022
History of risk factors				
Diabetes mellitus (%)	39.9	41.7	24.3	0.018
Hypercholesterolemia (%)	42.0	41.3	43.7	NS
Coronary heart disease (%)	20.2	19.7	21.4	NS
Atrial fibrillation	28.2	31.8	20.4	0.036
Family history for stroke (%)	24.8	24.7	25.2	NS
Stroke syndrome (%)				NS
TACS	10.4	10.8	9.7	
PACS	38.3	38.1	38.8	
LACS	20.0	19.3	21.4	
POCS	31.3	31.8	30.1	
Stroke etiology (%)				
Small-vessel occlusive	10.7	10.8	10.7	NS
Large-vessel occlusive	18.7	20.2	15.5	0.043
Cardioembolic	37.1	38.1	35.0	NS
Other	12.0	12.1	11.7	NS
Unknown	21.5	18.8	27.2	0.032
NIHSS at admission, (IQR)	8 (5–12)	9 (5–17)	4 (2–8)	<0.001
mRS at discharge (IQR)	1 (0–4)	2 (0–4)	1 (0–2)	<0.001

Results are expressed as percentages or as medians (IQR)

TACS total anterior circulation syndrome, *LACS* lacunar syndrome, *PACS* partial anterior circulation syndrome, *POCS* posterior circulation syndrome, *NIHSS* National Institutes of Health Stroke Scale, *mRS* modified Rankin scale

[a] Mann–Whitney U Test or χ^2 test were used

65 (IQR 57–75) years and 39.6 % were women. The median time from stroke onset to inclusion in the study was 6.8 (IQR 3.5–13.1) hours. The median hospital stay was 31(IQR 15–48) days. The median NIHSS score on admission was eight points (IQR 5–12). An unfavorable functional outcome was found in 118 patients (36.2 %) with a median mRS score of 4 (IQR 3–6). 38 patients died, thus the mortality rate was 11.7 %. Baseline characteristics in patients with AIS are provided in Table 1. One hundred and two patients (31.3 %) received thrombolytic therapy with rtPA within the first 3 h of patient admission.

Serum 25(OH) D Levels and Stroke Characteristics

In our study population, almost two-thirds of the patients showed deficiency levels of 25(OH) D.

The median serum level of 25(OH) D in patients with AIS was 14. 3 (IQR 10.1–21.5) ng/ml. Levels of serum 25(OH) D were compared based on 4 seasons of blood sampling. Significant seasonal differences in 25(OH) D levels were observed [analysis of variance (ANOVA): $P = 0.004$]. There was a negative correlation between levels of 25(OH) D and the hospital stay [r (spearman) $= -0.134$, $P = 0.032$].

The results indicated that the serum 25(OH) D levels gradual decline with increasing age [r (spearman) $= -0.157$, $P = 0.004$; See the Fig. 1a]. There was no correlation between serum 25(OH) D levels and sex ($P = 0.458$). In addition, serum 25(OH) D levels reduced with increasing severity of stroke as defined by the NIHSS score. There was a negative correlation between levels of 25(OH) D and the NIHSS (r $= -0.389$, $P < 0.0001$; Fig. 1b). There was still a significant negative trend between serum 25(OH) D levels and NIHSS score ($P = 0.012$), using ordered logistic regression after multivariate adjustment for possible confounders: age, hospital stay, seasons, risk factors, stroke subtype and syndrome. In patients for whom MRI data were available (n = 181), there was a negative correlation between levels of 25(OH) D and the infarct volume (r $= -0.355$, $P < 0.0001$; Fig. 1c).

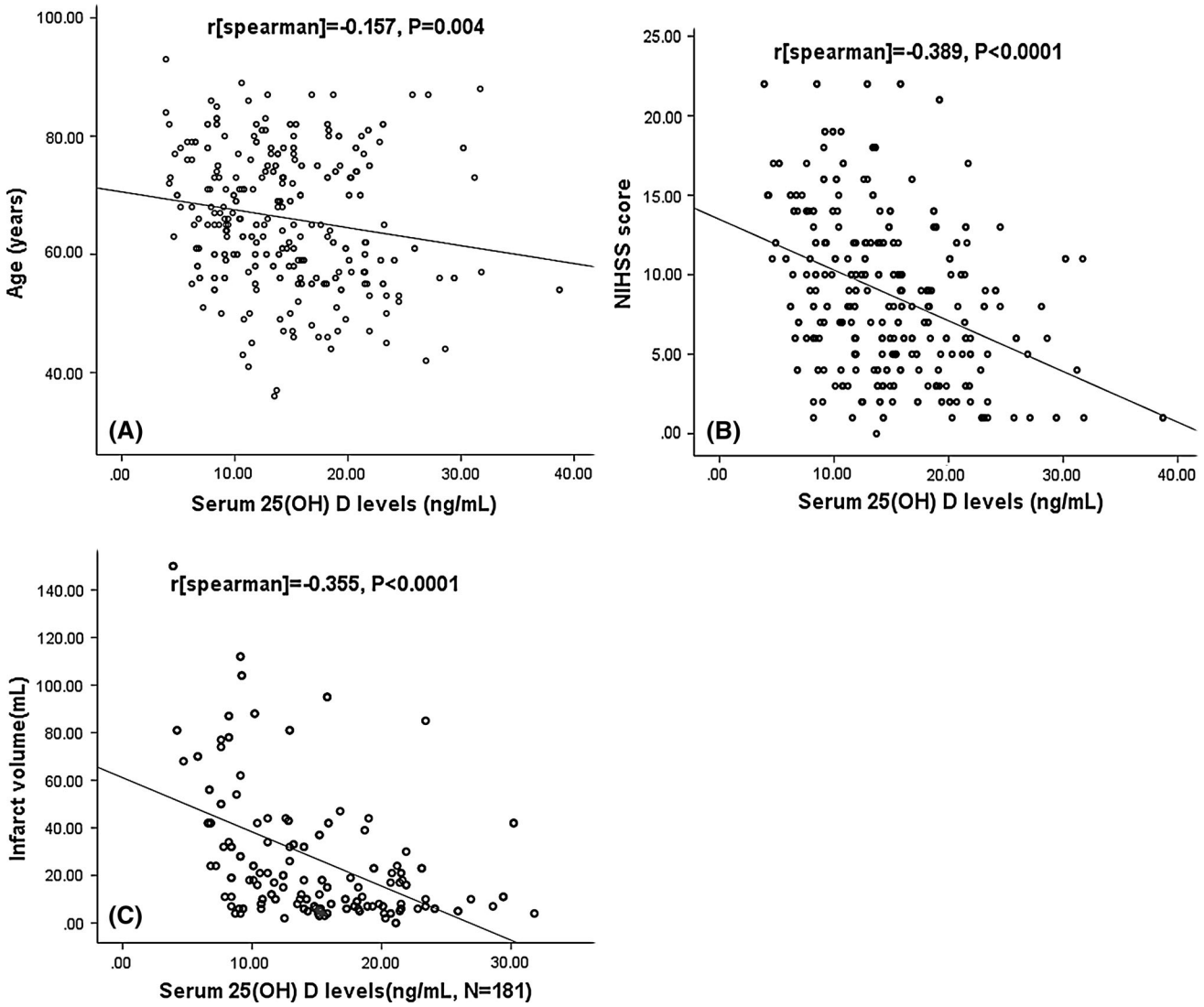

Fig. 1 Correlation between serum 25(OH) D levels and others predictors. **a** Correlation between serum 25(OH) D levels and age; **b** correlation between the serum 25(OH) D levels and the National Institutes of Health Stroke Scale (NIHSS) score; **c** correlation between serum 25(OH)D levels and infract volume

Serum 25(OH) D Levels and Stroke Outcome

Serum 25(OH) D levels in patients with a favorable outcome were significantly greater than those in patients with an unfavorable outcome [18.5 (IQR 13.8–23.1) vs 10.1 (IQR 8.2–13.1) ng/ml; $P = 0.000$; Fig. 2]. In univariate logistic regression analysis, we calculated the ORs of log-transformed 25(OH) D levels as compared with the NIHSS score and other risk factors. With an unadjusted OR of 6.13 (95 % CI 3.38 –12.25), 25(OH) D had a strong association with favorable outcome. After adjusting for all other significant outcome predictors, 25(OH) D remained can be seen as an independent favorable outcome predictor with an adjusted OR of 3.96 (95 % CI 2.85–7.87; $P = 0.000$).

Serum 25(OH) D levels in survived patients were significantly greater as compared with patients who died [15.3 (IQR 11.5–21.9) vs 9.2 (IQR 6.8–11.8) ng/ml; $P = 0.000$; See the Fig. 3]. After adjustment for other parameters, 25(OH) D levels remained an independent predictor for survival with an OR of 3.36 (95 % CI 2.12–7.08; $P = 0.000$).

Discussion

A large body of evidence from epidemiological studies indicates that vitamin D deficiency is associated with an increased risk of stroke [2, 4], and Stepwise decreasing plasma 25(OH) D concentrations were associated with a stepwise increasing risk of ischemic stroke [11]. In this study, we assessed the serum 25(OH) D levels with regard to their accuracy to predict functional outcome in patients

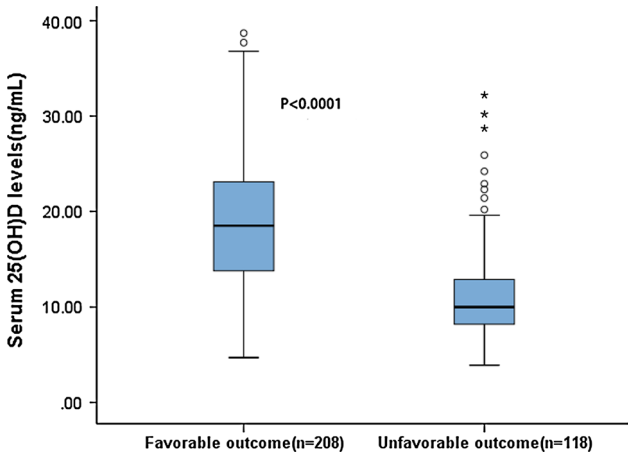

Fig. 2 Serum 25(OH) D levels in acute ischemic stroke patients with favorable and unfavorable outcome. Mann–Whitney U-test. All data are medians and interquartile ranges (IQR). Significantly higher in stroke patients with favorable outcomes as compared to unfavorable ($P < 0.0001$)

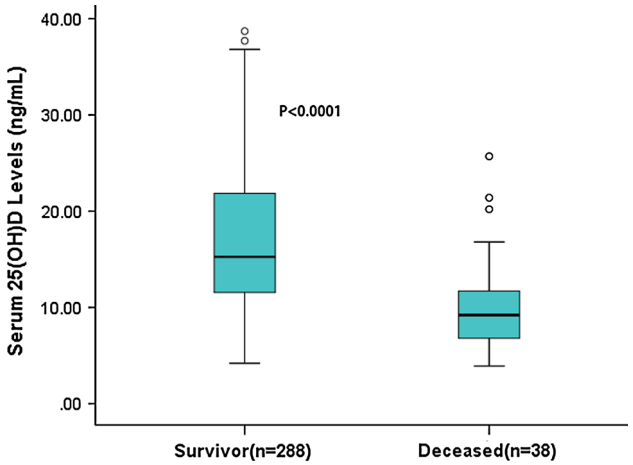

Fig. 3 Serum 25(OH) D levels in survivor and deceased patients with acute ischemic stroke. Mann–Whitney U-test. All data are medians and interquartile ranges (IQR). Significantly higher in survivor stroke patients as compared to deceased ($P < 0.0001$)

with AIS at discharge in Chinese population. Our main finding was that 25(OH) D can be seen as an independent prognostic marker of functional outcome in Chinese patients with AIS even after correcting for possible confounding 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).

These results are in accordance with the results from other studies. Daubail et al. [12] reported that a low serum 25(OH) D level is a predictor of both severity at admission and poor early functional outcome in stroke patients. Similarly, Tu et al. [19] suggested that 25(OH) D is an independent prognostic marker for death and functional outcome within 90 days in Chinese patients with AIS even after adjusting for possible confounding factors. Michos et al. [10] found that vitamin D deficiency was associated with an increased risk of stroke death in whites but not in blacks.

Interestingly, in our study, we found that circulating serum 25(OH) D levels were deficient in 68.4 % of patients with AIS. Another study in Chinese patients also found that Vitamin D deficiency (78.6 %) was very common [19]. Significant seasonal differences in 25(OH) D levels were observed in our study, which suggesting that different sunshine may affect the serum levels of 25(OH) D. In addition, the median serum level of 25(OH) D in our patients was 14.3 ng/ml, while was 14.2 ng/ml in another study [19]. This is very interesting because despite using different methods of assessment, sample size limitations, and potential dietary and genetic confounders, a similar prevalence of vitamin D deficiency in AIS patients was obtained, indicating a common phenomenon of vitamin D deficiency in Chinese patients with AIS.

The mechanism of the 25(OH) D and functional outcome in patients with AIS remains unclear. It has been suggested that vitamin D has neuroprotective properties [20], and vitamin D supplementation could contribute to reducing the volume of cerebral infarct in animal models of stroke [21]. Consistently, we found an inverse correction between infarct volume and 25(OH) D levels. Secondly, 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 [22]. Thirdly, some data also indicate that vitamin D deficiency may contribute to systemic inflammation [23]. Low 25(OH) D levels are known to influence macrophage and lymphocyte activity in atherosclerotic plaques and to promote chronic inflammation in the artery wall [24]. Various studies suggest that vitamin D may exert anti-inflammatory effects [25]. Reduced 25(OH) D might be associated with overall increased inflammatory activity [26].

Some limitations of this observational study merit consideration. Firstly, without serial measurement of the serum circulating 25(OH) D levels, this study yielded no data regarding the change of levels in these patients. Secondly, the present findings could not be extrapolated to all AIS patients, but may apply only to this selected subpopulation. Thirdly, we did not collect data on sun exposure and diet, so we could not determine the association of those factors with 25(OH) D levels. Thirdly, the observational nature of study made it impossible to determine causality of the observed associations and residual confounding by other

factors such as poorer health status could explain our findings. Lastly, novelty of this study is not very strong; however, study diversity is also very important and meaningful.

Conclusions

In conclusion, these results suggest that serum lower 25(OH) D levels can be seen as an independent prognostic marker of unfavorable functional outcome in Chinese patients with AIS. Whether vitamin D supplementation at adequate doses can improve prognosis in Chinese AIS patients urgently need to evaluate.

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