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ORIGINAL ARTICLE

A Prospective Study of Vitamin D Deficiency in Patients with Hemorrhagic Stroke

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Background: There is accumulating evidence that Vitamin D deficiency contributes to the occurrence of stroke, including ischemic and hemorrhagic strokes. However, the relationship between Vitamin D levels and the risk of hemorrhage stroke was less conclusive. **Aim:** This prospective study is aimed for relationship between Vitamin D status and specific nonlobar hemorrhagic stroke in a Taiwanese cohort. **Methods:** A prospective study of 44 adult patients (32 males and 12 females; 27 aged <65 years and 17 ≥65 years) with acute nonlobar spontaneous intracerebral hemorrhage (sICH) was undertaken for 24 months (December 2017–November 2019) in a general reference teaching hospital. The serum 25-hydroxyvitamin D (25(OH) D) level was examined within 1 day of the stroke. The associations between Vitamin D status, age, low-density lipoprotein levels, and hemorrhagic stroke were analyzed using the Chi-squared test for comparisons. Statistical significance was set at $P < 0.05$. **Results:** The mean serum concentration of (25(OH) D) was 20.30 ng/ml. There were 14 patients with Vitamin D insufficiency (<30 ng/ml), and 23 with Vitamin D deficiency (<20 ng/ml). There was no age dependence to the Vitamin D deficiency and insufficiency in patients with acute nonlobar sICH. We also found no significant correlation between Vitamin D deficiency and low-density lipoprotein concentration. **Conclusion:** A particularly high prevalence of Vitamin D deficiency was found in Taiwanese patients with specific hemorrhagic stroke, acute nonlobar sICH, and this was independent of age or serum low-density lipoprotein levels.

Key words: Vitamin D deficiency, hemorrhagic stroke, calcifediol, cerebral hemorrhage

INTRODUCTION

The hemorrhagic stroke, spontaneous intracerebral hemorrhage (sICH), is the second-most common form of stroke, and is associated with a significantly high morbidity and mortality.[1] The major causes include hypertension, cerebral amyloid angiopathy, vascular malformation, brain neoplasm, misuse of drugs or alcohol, pregnancy, and inherited or acquired coagulopathies.[2,3] Primary sICH is defined in the absence of any underlying vascular malformation or coagulopathy, trauma, or surgery. Primary sICH can be classified as either lobar or nonlobar, depending on the location of the intraparenchymal hematoma.[4,5] Lobar sICH is highly related to the deposition of amyloid in cortical perforators, which contribute to cerebral amyloid angiopathy formation, and consequent lobar hemorrhage.[6] The most common locations of nonlobar primary sICH are the basal ganglia, thalamus, pons, and cerebellum. Factors such as lipohyalinosis formation cause fragility of small perforating arteries in these regions.[5,7]

Although recent observational studies have shown a relationship between Vitamin D status and stroke risk, the results are inconsistent. In the meta-analysis result of the VITAL trial (Vitamin D and OmegA-3 TriaL), regular supplementation with Vitamin D_3 at a dose of 2000 IU/day was not effective for the primary prevention of ischemic or hemorrhage stroke.[8] In another population-based study, stroke was associated only with severe Vitamin D deficiency (<12 ng/ml or 30 nmol/l).[9] However, a recent review suggested less severe Vitamin D deficiency was a risk factor for stroke,[10] but many studies included more patients with ischemic stroke. The relationship between Vitamin D levels and the risk of hemorrhage stroke was less conclusive. Thus, we performed a

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prospective study of Vitamin D status and the risk of a specific hemorrhage stroke type, namely acute nonlobar primary sICH.

MATERIALS AND METHODS

We performed a prospective clinical study for 24 months (December 2017–November 2019) in a general reference teaching hospital (Tri-service General Hospital, Taipei, Taiwan). The study was approved by the human research ethics committee in the institution (2-106-05-135).

Adult patients, aged more than 20 years, were enrolled on presentation of acute nonlobar sICH in basal ganglia, thalamus, pons, or cerebellum within 1 day. The sICH was defined as an intraparenchymal hemorrhage in the absence of head trauma or brain surgery. The diagnosis was based on clinical presentation and brain computed tomography scans. Exclusion criteria were underlying malignancy, regular use of anticoagulants, or antiplatelet drugs before sICH, cerebral vascular malformation or aneurysm, systemic disease contributing to bleeding tendency, autoimmune disease, pregnancy, cigarette smoking, frequent alcohol use (>2 drinks/day), diabetes, brain surgery, previous stroke, and myocardial infarction. All patients or their families were provided with written informed consent for participation in the study by the principal investigator, and the researchers assessed the relationship between Vitamin D status and hemorrhage stroke from their medical information.

The level of 25-hydroxyvitamin D (25(OH) D) in serum was measured within 24 hours of the occurrence of neurological impairment with a Cobas 8000 modular analyzer, e801 (Roche Diagnostics, Mannheim, Germany), which uses an automated immunoassay based on streptavidin-biotin interactions.[11] Normal Vitamin D status was defined as ≥30 ng/ml (75 nmol/l), insufficiency as <30 ng/ml (75 nmol/l), and deficiency as <20 ng/ml (50 nmol/l) according to the National Academy of Medicine in the United States and the Endocrine Society clinical practice guideline.[9,12] The patients with acute sICH were then allocated to one of three groups based on their Vitamin D levels (normal, insufficient, or deficient).

We also collected complete laboratory profiles for patients in this study within 24 hours. The laboratory examinations included the hemoglobin level, platelet count, white blood cell count, plasma levels of urea, creatinine, aspartate aminotransferase, alanine aminotransferase, sodium, potassium, calcium, and albumin (as renal and liver function tests). The serum total cholesterol, triglyceride (TG), and high- and low-density lipoprotein (HDL and LDL) levels were also evaluated.

In patients younger than 65 years of age, the additional rheumatoid factor, C3, C4, antinuclear antibody, anti-cardiolipin antibodies, lupus anticoagulant, anti-HIV antibody, and rapid plasma reagin tests were performed to rule out autoimmune disease which may have contributed to sICH directly or indirectly.

Statistical analysis

The associations between Vitamin D status, age, LDL levels, and sICH were analyzed with the Chi-squared test for comparisons. Statistical significance was set at $P < 0.05$ for all analyses. Analyses were performed using SPSS 21.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

The demographic characteristics of patients are shown in Table 1. A total of 44 patients with sICH were enrolled, including 32 males and 12 females, 27 aged <65 years and 17 ≥65 years. The mean serum concentration of 25 (OH) D was 20.30 ng/ml. There were 14 patients with Vitamin D insufficiency, and 23 with Vitamin D deficiency. The mean serum LDL was 109.05 mg/dL. However, two enrolled patients did not complete the blood lipid examinations.

We compared the prevalence of Vitamin D deficiency in our study to the population-based vitamin status study of Taiwan.[13] The results are presented in Table 2. There was a higher prevalence of Vitamin D deficiency in our cohort of sICH patients (55.56% in aged <65 years and 47.06% in aged ≥65 years) compared with the population study (22.4% in the general Taiwan population, 26% in aged 30–59 years, 10% >60 years).[13] There was no age dependence on either Vitamin D deficiency or Vitamin D insufficiency in our cohort [Tables 3 and 4].

The mean LDL concentration was 105.32 mg/dL in the sICH patients without Vitamin D deficiency and 112.13 mg/dL in those with Vitamin D deficiency, which indicates no correlation between Vitamin D deficiency and LDL concentration [Table 5].

Table 1: Patient characteristics, Vitamin D levels, low-density lipoprotein concentrations

Variable	*n* (%)/mean±SD
Gender	
Male	32 (72.73)
Female	12 (27.27)
Age (years)	60.75±14.81
<65	27 (61.36)
≥65	17 (38.64)
Mean Vitamin D (ng/mL)	20.30±7.44
Vitamin D insufficiency	14 (31.82)
Vitamin D deficiency	23 (52.27)
Mean LDL (mg/dL)	109.05±32.62

LDL=Low-density lipoprotein; SD=Standard deviation

Table 2: Age and Vitamin D deficiency: Comparison with Taiwanese cohort study

Age (years)	*n*	Vitamin D deficiency, *n* (%)	P[†]
<65	27	15 (55.56)	<0.001
≥65	17	8 (47.06)	0.015

[†]Compare to reference 13: Lee MJ, Hsu HJ, Wu IW, Sun CY, Ting MK, Lee CC. Vitamin D deficiency in northern Taiwan: A community-based cohort study. BMC Public Health 2019;19:337. By using one-sample Chi-square test

Table 3: Age and Vitamin D deficiency

Age (years)	*n*	Vitamin D deficiency, *n* (%)	P[§]
<65	27	15 (55.56)	0.583
≥65	17	8 (47.06)	

[§]Chi-square test

Table 4: Age and Vitamin D insufficiency

Age	*n*	Vitamin D insufficiency, *n* (%)	P[§]
<65	27	8 (29.63)	0.694
≥65	17	6 (35.29)	

[§]Chi-square test

Table 5: Vitamin D deficiency and low-density lipoprotein levels

Vitamin D deficiency	*n*	LDL (mg/dL)	P[§]
No	19	105.32 (35.28)	0.507
Yes	23	112.13 (30.71)	

[§]Mean±SD, Independent *t*-test. LDL=Low-density lipoprotein; SD=Standard deviation

DISCUSSION

In this prospective observational study, we found that low serum 25(OH) D levels are highly related to primary nonlobar sICH. This association was independent of traditional hemorrhagic stroke risk factors due to rigorous exclusion criteria. These results indicate that lower 25(OH) D is an important risk factor for nonlobar hemorrhagic stroke.

In the population-based study in Taiwan, the mean 25(OH) D concentration was 28.94 ± 10.27 ng/ml, and 22.4% (7.2%–38.4%) had Vitamin D deficiency.[13] The prevalence of Vitamin D deficiency in our study from a similar geographical area was much higher (55.56% in sICH patients aged <65 years and 47.06% in aged ≥65 years). Vitamin D deficiency rates of 65.7% in males and 76.7% in females were reported from the Korea National Health and Nutrition Examination Survey conducted in 2014.[14] The International Federation of Clinical Chemistry Global Multicenter study of reference intervals in China found the prevalence rate of Vitamin D deficiency was 55.9%, significantly higher in women, and less associated with latitude. [15] The percentage of intracerebral hemorrhage accounting for all strokes has been reported higher in South Korea (23.9%) and China (23.4%) than that in Taiwan (16.1%).[16]

Lipids are important components of cell membranes that stabilize the endothelial cells and maintain the integrity of cerebral small vessels. Dyslipidemia, hyperlipidemia, or hypolipidemia are risk factors for sICH.[5,17] Lower TG has been reported as a risk factor for sICH, especially if the serum TG level was ≤0.94 mmol/l (83.26 mg/dL).[18,19] However, in one population-based multivariate analysis study, the serum TG level was correlated positively with the incidence of sICH.[20] We found no sICH patient in our study with a low TG level ≤83.26 mg/dL, but seven patients (15.91%, 5 in aged <65 years and 2 in ≥65 years) were found to have hyperlipidemia (LDL >160 or TG >175 mg/dL, "2018 Guideline on the Management of Blood Cholesterol").[21] In addition, the cohort study conducted by Ma *et al*. showed that the lower LDL-C was associated with a significantly higher risk of sICH, especially if LDL-C was <70 mg/dL.[22] This was confirmed in a meta-analysis. [23] In our study, the mean LDL level was 109.05 mg/dL, and we found only five patients (11.36%, 2 in aged <65 years and 3 in ≥65 years) with LDL-C <70 mg/dL.

In a Chinese cohort study of 3788 patients, Vitamin D deficiency was associated with dyslipidemia. There were negative correlations between serum 25(OH) D and LDL or TG levels, and a positive correlation with HDL levels. [24] However, this association was not found in our study.

The strength of this study is its prospective nature with the exclusion of traditional cerebrovascular disease risk factors. However, the current study cannot explain clearly the mechanism linking low Vitamin D levels and this type of hemorrhagic stroke. The major cause to Vitamin D deficiency is inadequate sunlight exposure. [25,26] The 7-dehydrocholesterol in the plasma membrane of epidermal cells absorb the UVB radiation with wavelengths of 290–315 nm during sun exposure, and this results in production of the conformation of s-cis, s-cis-previtamin D_3, and s-trans, s-cis previamin previtamin D_3. The s-cis, s-cis-previtamin D_3 is converted to Vitamin D_3 and then transformed to 25(OH) D in the liver. The decreased concentration of 7-dehydrocholesterol has been reported in the elderly population and an increasing prevalence of Vitamin D deficiency with advancing age has been demonstrated in many studies.[27,28] However, in our study, the Vitamin D deficiency was not related to old age in patients with sICH.

Very few natural foods contain Vitamin D. Regular supplementation with Vitamin D_3 has not caused a lower incidence of major cardiovascular events.[8] The use of Vitamin

D_2 is less potent, and may even induce the destruction of 25(OH) D and consequent Vitamin D deficiency.[29] A higher dietary Vitamin D intake has been suggested to reduce the risk of stroke mortality, especially hemorrhagic stroke,[30] but will require large trials to prove a significant beneficial effect.

There are several limitations to our study. First, the number of enrolled sICH patients was not large enough to identify and analyze a significant relationship. Second, we did not have details of sun exposure, dietary habits, body mass, occupation, educational status, the season of the sICH occurrence, residential district, and other relevant factors.[13,31,32] Third, the blood sampling, storage at −80°C, and delay in measurement of 25(OH) D measurement with an immunoassay module may have caused less precise 25(OH) D values than the gold standard technique (liquid chromatography-tandem mass spectrometry), and perhaps resulted in an apparent increase in Vitamin D deficiency.[33] Finally, two sICH patients did not complete blood lipid examinations.

CONCLUSION

In this study, we demonstrated a particularly high prevalence of Vitamin D deficiency in patients with the specific hemorrhagic stroke, nonlobar sICH, and this was independent of age and blood lipid profile. This may have implications for education and for preventing hemorrhagic stroke.

Data availability statement

The data that support the findings of this study are available from the corresponding author, KN Chou, upon reasonable request.

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Conflicts of interest

There are no conflicts of interest.

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