The combined presence of hypertension and vitamin D deficiency increased the probability of the occurrence of small vessel disease in China

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Junzeng Si
qilu hospital of shandong university

kuibao li
Beijing Chaoyang Hospital

peiyan shan
qilu hospital of shandong university

Junliang Yuan ✉ yuan_doctor@163.com
Beijing Chaoyang Hospital
Corresponding Author

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Abstract

Background: The exact relationship between 25-hydroxyvitamin D 25(OH)D levels and small vessel disease (SVD) are not clear in China. The aim of this study was to determine the association between 25(OH)D and SVD in China. Methods: We enrolled consecutive 106 patients with SVD and 115 controls in Beijing Chaoyang Hospital and Jinan City people’s hospital between Jan 2017 and Dec 2017. Vitamin D status was estimated by measuring serum 25-hydroxyvitamin D 25(OH)D. The subjects were categorized into three subgroups: vitamin D deficiency (≤12ng/ml), insufficiency (12-20ng/ml) and sufficiency (≥20 ng/ml). Results: Among 106 stroke patients, 80 (75.5%) were men and mean age was 61.6±13.2 years. 25(OH)D deficiency was observed in 76 (71.7%) stroke patients and 47 (40.9%) controls (P=0.001). Comparing with controls, patients with SVD were correlated with higher proportion of male, the histories of stroke, smoking and hyperlipidemia; higher systolic blood pressure, diastolic blood pressure and low density lipoprotein; lower of 25(OH)D level. The logistic regression analysis showed the level of 25(OH)D was independently predicted the occurrence of SVD (OR 0.772, CI 0.691-0.862, P=0.001). Compared with those in sufficiency group (≥20ng/ml), the ORs of SVD in the deficiency (≤12ng/ml) and insufficiency group (12-20 ng/ml) of 25(OH)D were 5.609 95% confidence interval (CI) 2.006-15.683 and 1.077 (95% CI: 0.338-3.428) after adjusting for potential confounders, respectively. We also found a significant effect modification of SVD risk by 25(OH)D status and hypertension interaction (P=0.001), and compared with those with sufficiency 25(OH)D levels, in hypertensives with vitamin D deficiency (≤12ng/ml) and insufficiency (12-20 ng/ml), the ORs were increased to 9.738 (2.398-39.540) and 1.108 (0.232-5.280), respectively (Pinteraction=0.001). Conclusion: Our findings showed patients with SVD were correlated with the deficiency of 25(OH)D. The combined presence of hypertension and vitamin D deficiency increased the probability of developing SVD. Our
study raises the importance that vitamin D supplementation combined with monitoring hypertension are promising approaches in the management of SVD.

Background

Stroke is now recognized to be the second leading cause of death worldwide [1, 2] and the first one in China [3]. Small vessel disease (SVD) is considered to cause 20% to 25% of strokes. There are several neuroimaging features of SVD according to the STandards for ReportIng Vascular changes on nEuroimaging (STRIVE), which include acute small subcortical infarcts, white matter hypertensities (WMHs), lacunes, microbleeds (CMBs), perivascular spaces (PVS) and brain atrophy [4]. To date, SVD has been recognized to be the second commonest cause of dementia, cognitive decline, physical frailty and late onset depression [5]. SVD is becoming a major public health problem in developing countries especially in China.

However, SVD is not silent, permanent or untreatable. Identification of controllable and treatable risk factors is essential for effective prevention of SVD. Importantly, accumulating evidences have opened new insights and offered new therapeutic targets in recent years. As a fat-soluble vitamin, an increasing body of evidences supported a vital role for vitamin D in brain function and development. Vitamin D may also prevent vascular injury through lowering blood pressure, inhibiting the renin-angiotensin-aldosterone system and inhibiting atherogenesis [6]. The prevalence of 25-hydroxyvitamin D [25(OH) D] deficiency is high in patients with acute stroke, and it may be associated with greater clinical severity and poor functional prognosis. However, there are only quite a few reports about the exact relationship between 25(OH) D and the occurrence of SVD. It was reported that 25(OH)D was inversely associated with lacunes, WMHs, and deep CMBs, which was linked to chronic brain injury associated with SVD in Korea [7]. The findings from India also indicated the combined presence of hypertension and vitamin D deficiency
increased the probability of developing vascular dementia (VaD) due to SVD, and the intervention of vitamin D status and hypertension could be helpful to reduce the risk of VaD due to SVD in Asian Indian subjects [8]. However, whether these findings could be reproduced in other ethnicities should be confirmed.

To date, only one study from China was reported an association between vitamin D deficiency and total MRI burden of cerebral small vessel disease in south of China [9]. Whether the effect of 25(OH)D on SVD is modified by the history of hypertension needs further to be elucidated in China. We hypothesized that there might be some relationships between 25(OH)D deficiency and the occurrence of SVD in north of China. Thus, in our present study, we aimed to investigate the exact relationship between 25(OH) D levels and the predictors of SVD in China.

Methods

Subjects

We enrolled consecutive 106 patients with small vessel disease (SVD) and 115 controls in department of neurology, Beijing Chaoyang Hospital and Jinan City people’s hospital between Jan 2017 and Dec 2017. The demographic data, clinical features, and laboratory data were analyzed. Magnetic resonance imaging (MRI) was performed to determine the presence of SVD, including lacunar infarction, lacunes, WMHs, CMBs and PVS according to STRIVE [4]. The lacunar infarction, lacunes and WMHs were identified from diffusion weighting imaging and fluid attenuated inversion recovery sequences, and microbleeds from subtraction weighted imaging. Our work was approved by the Ethics Committee of Beijing Chaoyang Hospital and Jinan City people’s hospital. Written informed consent was obtained from all participants.

Clinical Variables

We obtained the following clinical data: age, gender; vascular risk factors such as
hypertension, diabetes mellitus, hyperlipidemia, stroke, coronary heart disease, hyperlipidaemia, atrial fibrillation, peripheral arterial disease and current smoking. The laboratory blood tests were obtained including the counts of red blood cell, white blood cell, platelet, fibrinogen, fasting blood glucose, hemoglobin A1c, glycated albumin, uric acid, homocysteine, total cholesterol, low-density lipoprotein, high-density lipoprotein, triglyceride, C-reactive protein, albumin, 25-hydroxy vitamin D. As for the Vitamin D level, serum levels of 25(OH)D were measured using enzyme immuno-assay kits. The subjects were stratified into vitamin D deficiency [25(OH)D: \( \leq 12 \) ng/ml], insufficiency [25(OH)D: 12-20 ng/ml] and sufficiency [25(OH)D: \( \geq 20 \) ng/ml] groups [8, 10] according the guidelines of the National Osteoporosis Society [10].

**Statistical Analysis**

The data were described using the mean and standard deviation values for continuous variables, the median and interquartile range values for categorical variables, and absolute numbers and percentages for nominal and categorical variables, and we compared the groups using the nonparametric Mann-Whitney U test. We performed a chi-square test to determine the correlation between categorical variables and a t test between continuous variables. The association between 25(OH)D and SVD was tested using logistic regression analyses. The odds ratios (ORs) and 95% confidence intervals (CIs) were estimated by binary logistic regression analysis. We used the Statistical Package for Social Sciences (SPSS) version 16.0 (SPSS Inc., Chicago, IL, USA) for data analysis. A P value less than 0.05 was considered statistically significant.

**Results**

A total of 221 subjects were enrolled, and 106 were patients with SVD and 115 for controls. Among 106 stroke patients, 80 (75.5%) were men and mean age was 61.6±13.2 years. The levels of 25(OH)D were classified as sufficient in 11 patients (10.4%) vs 25
controls (21.7%), insufficient in 19 patients (17.9%%) vs 43 controls (37.4%), and
deficient in 76 patients (71.7%) vs 47 controls (40.9%). There was statistically significant
of vitamin D deficiency in SVD compared to controls (P=0.001).
The demographics, vascular risk factors, and the laboratory findings between the two
groups were presented in Table 1. Comparing with controls, patients with SVD were
correlated with male, higher proportion of histories of stroke, smoking and
hyperlipidaemia, higher systolic blood pressure, diastolic blood pressure and low density
lipoprotein (P<0.05). The mean serum 25(OH)D level was lower in SVD patients (10.2±5.6
ng/ml) compared with controls (14.6±7.5ng/ml), P=0001.
The binary logistic regression analysis showed the level of 25(OH)D was independently
predicted the occurrence of SVD (OR 0.772, CI 0.691-0.862, P=0.001) after adjusting for
the other covariates, which was showed in Table 2. Compared with those in sufficiency
group (≥20ng/ml) of 25(OH)D, we also found that the ORs of SVD in the deficiency
(≤12ng/ml) and insufficiency group (12–20 ng/ml) were 5.609 [95% CI 2.006-15.683] and
1.077 (95% CI 0.338-3.428) after adjusting for potential confounders, respectively (Table
3).
We also found that there were a significant interaction on presence of SVD between
vitamin D status and hypertension, and in hypertensives with vitamin D deficiency
(≤12ng/ml) and insufficiency (12-20 ng/ml), the ORs were increased to 9.738 (2.398-
39.540) and 1.108 (0.232-5.280), respectively (Pinteraction=0.001) (Table 4). However, we
did not find such interaction between vitamin D status and hypertension in non-
hypertensive patients (P=0.257 for model 1 and P=0.075 for model 2).
Discussion
In our present study, we found patients with SVD were correlated with the deficiency of
25(OH)D. We also found that there was a significant interaction between vitamin D status and hypertension in patients with SVD.

SVD is a common cause of stroke and worsens all stroke outcomes. SVD is associated with vascular risk factors such as hypertension. As a neurosteroid, vitamin D may play a vital role in preventing vascular injury through the mechanisms of lowering blood pressure, inhibiting the renin-angiotensin-aldosterone system, and inhibiting atherogenesis [6]. Accumulating evidences have also supported the hypothesis that lower 25(OH)D level was associated with increased risk of ischemic stroke, higher mortality and poor functional outcome in different races or ethnicities [11-22], and higher level of vitamin D was linked with a decreased risk of cerebrovascular disease [23]. These findings were also substantiated from meta-analysis [24]. However, as for the SVD, the relationship between vitamin D status and SVD has been still uncertain [25]. Recently, some cross-sectional studies proved that 25(OH)D was inversely associated with lacunes, WMHs and deep CMBs [7]. The findings from India also revealed that deficient levels of vitamin D were associated with 2.2-fold increase in odds of VaD due to SVD [8]. As for the total burden of SVD, lower levels of 25(OH)D was associated with greater total SVD burden seen on MRI in ischemic stroke [9]. However, the above studies had limited representation of the individuals from China. In our study, we further confirmed patients with SVD were correlated with the deficiency of 25(OH)D. Our study was in line with the former studies in different race such as in in Nepalese population or India [11, 26-30].

Hypertension is considered as an important, preventable risk factor for cardiovascular and stroke. There is a growing body of evidences about the association between vitamin D and hypertension in different ethnicities. In Asian Indian population, an inverse association was found between 25(OH)D and risk of ischemic stroke, which indicated that management of hypertension and treatment of severe vitamin D deficiency particularly in
hypertensive subjects could be helpful to prevent stroke effectively [31]. Another observational studies also supported a relationship between 25(OH)D, a higher blood pressure and ischemic stroke [32]. Our results are consistent with the former studies [8, 31], with the evidence that presence of hypertension may amply aggravate the risk of SVD with low vitamin D levels [33]. Our findings also seem to be in some accordance with the evidence of cardiovascular disease from our research team, with the results that presence of hypertension may modify the association of vitamin D deficiency with severity of coronary stenosis [34]. As known, both hypertension and vitamin D deficiency are controllable and treatable parameters, thus, monitoring and management of vitamin D and hypertension may reduce the risk of SVD in China. Random clinical trials should also focus on supplementation of vitamin D in subjects with vitamin D deficiency and particularly those with a history of hypertension.

However, the mechanism of deficiency of vitamin D and the developing of SVD is not fully understood. It has been reported that vitamin D may also play a role in neuroprotection, perhaps through detoxification pathways, stimulation of neurotrophic factors, inhibition of inducible nitric oxide synthase, antioxidation/anti-inflammatory, neuronal calcium regulation, enhanced nerve conduction, as well as antithrombotic properties [35]. Serum vitamin D levels were inversely associated with the levels of interleukin-6 and C reactive protein, suggesting a potential anti-inflammatory role for vitamin D in stroke [36, 37]. It is also plausible that vitamin D supplementation could be a beneficial intervention for the prevention and treatment of stroke. Contributing mechanisms have been linked to the association of vitamin D deficiency with the presence of hypertension and atherosclerosis, however, the conclusions are still conflicting and data from large, randomized clinical trials are needed to clarify these speculations[38].

Our study also had some limitations. Firstly, this study was designed as a cross-sectional
study from two centers in north of China, however, we could not prove a causal relationship between 25(OH)D and SVD. Studies with a larger numbers from multiple centers in China are needed urgently to further confirm our findings. Secondly, the multiple other factors such as nutrition status, physical activity, additional vitamin D supplementation, the levels of serum calcium, phosphorus and parathyroid hormone, health education, social status, levels of inflammatory markers, seasonal categories (such as summer or winter), vitamin D receptor gene polymorphism were not available in our study [8, 31, 39]. However, these factors were also of utmost importance, and we could not adjust multivariable analysis for these variables. Thirdly, we did not classify SVD into different types according to neuroimaging such as lacunes, WMHs, CMBs or brain atrophy [7, 40]. It has been reported that the severity of SVD could be assessed by total SVD burden (0 to 4) [9]. Further studies are required to confirm this association and explore the association among different subtypes of SVD on neuroimaging [7, 13]. Fourthly, there were some different categories according to levels of vitamin D (stratified into 2 or 3 or 5 categories) in different studies [31, 41], such as deficiency (<25nmol/L or <20ng/mL), insufficiency (25–50nmol/L or 20–30ng/mL), sufficiency (≥50nmol/L or ≥ 30ng/mL) [37, 42, 43]. In spite of these limitations, to the best of our knowledge, this is the first study to report a link between vitamin D status and hypertension associated with SVD in Chinese population. However, our findings also need to be validated in a larger study in other regions in China.

Conclusions

Our study demonstrated that vitamin D deficiency significantly increases the risk of SVD. We also found that the combined presence of hypertension and vitamin D deficiency increased the probability of developing SVD. Our findings will warrant further prospective studies or large scale interventional studies in multiple centers with large samples in the
future.

Abbreviations

25(OH) D: 25-hydroxyvitamin D; SVD: small vessel disease; STRIVE: STAndards for ReporTIng Vascular changes on nEuroimaging; WMHs: hypertensities; CMBs: microbleeds; PVS: perivascular spaces; VaD: vascular dementia; OR: odds ratio; CI: confidence interval.

Declarations

**Ethics approval and consent to participate**

Our work was approved by the Ethics Committee of Beijing Chaoyang Hospital and Jinan City people’s hospital. Written informed consent was obtained from all participants.

**Consent for publication**

Not applicable.

**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author upon request.

**Competing interests**

The authors declare that they have no competing interests.

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**Authors’ contributions**

JLY and PYS conceived and designed the experiments. JZS analyzed the data and drafted
the manuscript. JZS and KBL collected data. All authors have read and approved the final manuscript to be published.

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References

1. Donkor ES: Stroke in the 21(st) Century: A Snapshot of the Burden, Epidemiology, and Quality of Life. Stroke research and treatment 2018, 2018:3238165.

2. Katan M, Luft A: Global Burden of Stroke. Seminars in neurology 2018, 38(2):208-211.

3. Wang W, Jiang B, Sun H, Ru X, Sun D, Wang L, Wang L, Jiang Y, Li Y, Wang Y et al: Prevalence, Incidence, and Mortality of Stroke in China: Results from a Nationwide Population-Based Survey of 480 687 Adults. Circulation 2017, 135(8):759-771.

4. Wardlaw JM, Smith EE, Biessels GJ, Cordonnier C, Fazekas F, Frayne R, Lindley RI, O'Brien JT, Barkhof F, Benavente OR et al: Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. Lancet Neurol 2013, 12(8):822-838.

5. Wardlaw JM: William M. Feinberg Award for Excellence in Clinical Stroke: Small Vessel Disease; a Big Problem, But Fixable. Stroke 2018, 49(7):1770-1775.

6. Michos ED, Melamed ML: Vitamin D and cardiovascular disease risk. Current opinion in clinical nutrition and metabolic care 2008, 11(1):7-12.

7. Chung PW, Park KY, Kim JM, Shin DW, Park MS, Chung YJ, Ha SY, Ahn SW, Shin HW, Kim YB et al: 25-hydroxyvitamin D status is associated with chronic cerebral small vessel disease. Stroke 2015, 46(1):248-251.
8. Prabhakar P, Chandra SR, Supriya M, Issac TG, Prasad C, Christopher R: Vitamin D status and vascular dementia due to cerebral small vessel disease in the elderly Asian Indian population. *J Neurol Sci* 2015, 359(1-2):108-111.

9. Feng C, Tang N, Huang H, Zhang G, Qi X, Shi F: 25-Hydroxy vitamin D level is associated with total MRI burden of cerebral small vessel disease in ischemic stroke patients. *Int J Neurosci* 2018:1-6.

10. Aspray TJ, Bowring C, Fraser W, Gittoes N, Javaid MK, Macdonald H, Patel S, Selby P, Tanna N, Francis RM: National Osteoporosis Society vitamin D guideline summary. *Age Ageing* 2014, 43(5):592-595.

11. Sun Q, Pan A, Hu FB, Manson JE, Rexrode KM: 25-Hydroxyvitamin D levels and the risk of stroke: a prospective study and meta-analysis. *Stroke* 2012, 43(6):1470-1477.

12. Chowdhury R, Stevens S, Ward H, Chowdhury S, Sajjad A, Franco OH: Circulating vitamin D, calcium and risk of cerebrovascular disease: a systematic review and meta-analysis. *Eur J Epidemiol* 2012, 27(8):581-591.

13. Zhou R, Wang M, Huang H, Li W, Hu Y, Wu T: Lower Vitamin D Status Is Associated with an Increased Risk of Ischemic Stroke: A Systematic Review and Meta-Analysis. *Nutrients* 2018, 10(3).

14. Kojima G, Bell C, Abbott RD, Launer L, Chen R, Motonaga H, Ross GW, Curb JD, Masaki K: Low dietary vitamin D predicts 34-year incident stroke: the Honolulu Heart Program. *Stroke* 2012, 43(8):2163-2167.

15. Pilz S, Tomaschitz A, Drechsler C, Zittermann A, Dekker JM, Marz W: Vitamin D supplementation: a promising approach for the prevention and treatment of strokes. *Curr Drug Targets* 2011, 12(1):88-96.

16. Witham MD, Dove FJ, Sugden JA, Doney AS, Struthers AD: The effect of vitamin D replacement on markers of vascular health in stroke patients - a randomised
controlled trial. *Nutr Metab Cardiovasc Dis* 2012, 22(10):864-870.

17. Park KY, Chung PW, Kim YB, Moon HS, Suh BC, Won YS, Kim JM, Youn YC, Kwon OS: Serum Vitamin D Status as a Predictor of Prognosis in Patients with Acute Ischemic Stroke. *Cerebrovasc Dis* 2015, 40(1-2):73-80.

18. Daumas A, Daubail B, Legris N, Jacquin-Piques A, Sensenbrenner B, Denimal D, Lemaire-Ewing S, Duvillard L, Giroud M, Bejot Y: Association between Admission Serum 25-Hydroxyvitamin D Levels and Functional Outcome of Thrombolyzed Stroke Patients. *J Stroke Cerebrovasc Dis* 2016, 25(4):907-913.

19. Thapa L, Shrestha A, Pradhan M, Bhandari TR, Shrestha S, Poudel RS, Poudel R, Pokhrel B: Status of Vitamin D and its Association with Stroke Risk Factors in Patients with Acute Ischemic Stroke in a Tertiary Care Hospital. *JNMA J Nepal Med Assoc* 2014, 52(195):935-939.

20. Qiu H, Wang M, Mi D, Zhao J, Tu W, Liu Q: Vitamin D Status and the Risk of Recurrent Stroke and Mortality in Ischemic Stroke Patients: Data from a 24-Month Follow-Up Study in China. *J Nutr Health Aging* 2017, 21(7):766-771.

21. Leung RY, Han Y, Sing CW, Cheung BM, Wong IC, Tan KC, Kung AW, Cheung CL: Serum 25-hydroxyvitamin D and the risk of stroke in Hong Kong Chinese. *Thromb Haemost* 2017, 117(1):158-163.

22. Schneider MA: The Importance of Educating Patients With Stroke About Vitamin D. *J Neurosci Nurs* 2017, 49(6):387-389.

23. Kilkkinen A, Knekt P, Aro A, Rissanen H, Marniemi J, Heliovaara M, Impivaara O, Reunanen A: Vitamin D status and the risk of cardiovascular disease death. *Am J Epidemiol* 2009, 170(8):1032-1039.

24. Brondum-Jacobsen P, Nordestgaard BG, Schnohr P, Benn M: 25-hydroxyvitamin D and symptomatic ischemic stroke: an original study and meta-analysis. *Annals of*
25. Carluccio MA, Di Donato I, Pescini F, Battaglini M, Bianchi S, Valenti R, Nannucci S, Franci B, Stromillo ML, De Stefano N et al: Vitamin D levels in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). *Neurol Sci* 2017, 38(7):1333-1336.

26. Pokharel BR, Kharel G, Thapa LJ, Rana PV: Vitamin D and Other Risk Factors among Stroke Patients. *Kathmandu Univ Med J (KUMJ)* 2015, 13(49):71-73.

27. Narasimhan S, Balasubramanian P: Role of Vitamin D in the Outcome of Ischemic Stroke- A Randomized Controlled Trial. *J Clin Diagn Res* 2017, 11(2):CC06-CC10.

28. Kim K, Cho KH, Im SH, Choi J, Yu J, Kim M: Decrement of Serum Vitamin D Level After Stroke. *Ann Rehabil Med* 2017, 41(6):944-950.

29. Pilz S, Dobnig H, Fischer JE, Wellnitz B, Seelhorst U, Boehm BO, Marz W: Low vitamin d levels predict stroke in patients referred to coronary angiography. *Stroke* 2008, 39(9):2611-2613.

30. Afshari L, Amani R, Soltani F, Haghighizadeh MH, Afsharmanesh MR: The relation between serum Vitamin D levels and body antioxidant status in ischemic stroke patients: A case-control study. *Adv Biomed Res* 2015, 4:213.

31. Majumdar V, Prabhakar P, Kulkarni GB, Christopher R: Vitamin D status, hypertension and ischemic stroke: a clinical perspective. *J Hum Hypertens* 2015, 29(11):669-674.

32. Afzal S, Nordestgaard BG: Vitamin D, Hypertension, and Ischemic Stroke in 116 655 Individuals From the General Population: A Genetic Study. *Hypertension* 2017.

33. Vimaleswaran KS, Cavadino A, Berry DJ, Jorde R, Dieffenbach AK, Lu C, Alves AC, Heerspink HJ, Tikkanen E, Eriksson J et al: Association of vitamin D status with arterial blood pressure and hypertension risk: a mendelian randomisation study. *The lancet Diabetes & endocrinology* 2014, 2(9):719-729.
34. Li K, Zhao W, Wang L, Yang X, Yang X: Effect modification of hypertension on the association of vitamin D deficiency with severity of coronary stenosis. *Blood pressure* 2018, 27(3):134-140.

35. Buell JS, Dawson-Hughes B: Vitamin D and neurocognitive dysfunction: preventing "D"ecline? *Molecular aspects of medicine* 2008, 29(6):415-422.

36. Wang Q, Zhu Z, Liu Y, Tu X, He J: Relationship between serum vitamin D levels and inflammatory markers in acute stroke patients. *Brain Behav* 2018, 8(2):e00885.

37. Alfieri DF, Lehmann MF, Oliveira SR, Flauzino T, Delongui F, de Araujo MC, Dichi I, Delfino VD, Mezzaroba L, Simao AN et al: Vitamin D deficiency is associated with acute ischemic stroke, C-reactive protein, and short-term outcome. *Metab Brain Dis* 2017, 32(2):493-502.

38. Makariou SE, Michel P, Tzoufi MS, Challa A, Milionis HJ: Vitamin D and stroke: promise for prevention and better outcome. *Curr Vasc Pharmacol* 2014, 12(1):117-124.

39. Supriya M, Chandra SR, Prabhakar P, Prasad C, Christopher R: Vitamin D receptor (VDR) gene polymorphism and vascular dementia due to cerebral small vessel disease in an Asian Indian cohort. *J Neurol Sci* 2018, 391:84-89.

40. Chaudhuri JR, Mridula KR, Alladi S, Anamika A, Umasamhesh M, Balaraju B, Swath A, Bandaru VS: Serum 25-hydroxyvitamin d deficiency in ischemic stroke and subtypes in Indian patients. *J Stroke* 2014, 16(1):44-50.

41. Zhang B, Wang Y, Zhong Y, Liao S, Lu Z: Serum 25-hydroxyvitamin D deficiency predicts poor outcome among acute ischemic stroke patients without hypertension. *Neurochemistry international* 2018, 118:91-95.

42. Cranney A, Horsley T, O'Donnell S, Weiler H, Puil L, Ooi D, Atkinson S, Ward L, Moher D, Hanley D et al: Effectiveness and safety of vitamin D in relation to bone health. *Evidence report/technology assessment* 2007(158):1-235.
Chen H, Liu Y, Huang G, Zhu J, Feng W, He J: Association between vitamin D status and cognitive impairment in acute ischemic stroke patients: a prospective cohort study. *Clin Interv Aging* 2018, 13:2503-2509.

**Tables**

Table 1. The baseline demographics, clinical characteristics and biochemical variables between the two groups
### Variables

#### Demographics

| Variable                        | SVD (N=106) | Control (N=115) | P       |
|---------------------------------|-------------|-----------------|---------|
| Age (Y)                         | 61.6±13.2   | 60.5±11.7       | 0.516   |
| Sex (Male, %)                   | 80(75.5%)   | 54(47%)         | 0.001*  |
| Systolic blood pressure         | 151.5±21    | 133.7±17.3      | 0.001*  |
| Diastolic blood pressure        | 87±14.2     | 80.1±12.3       | 0.001*  |

#### Risk factors

| Variable                        | SVD          | Control       | P       |
|---------------------------------|--------------|---------------|---------|
| Hypertension                    | 66(62.3%)    | 75(65.2%)     | 0.648   |
| Diabetes mellitus               | 33(31.1%)    | 26(22.6%)     | 0.152   |
| Coronary disease                | 11(10.4%)    | 16(13.9%)     | 0.423   |
| Prior stroke                    | 26(24.5%)    | 5(4.3%)       | 0.001*  |
| Hyperlipidaemia                 | 79(74.5%)    | 31(27%)       | 0.001*  |
| Atrial fibrillation             | 2(2.3%)      | 2(6.7%)       | 0.251   |
| Peripheral arterial disease     | 6(5.7%)      | 3(2.6%)       | 0.252   |
| Smoking                         | 60(56.6%)    | 45(39.1%)     | 0.010*  |

#### Blood tests

| Variable                        | SVD          | Control       | P       |
|---------------------------------|--------------|---------------|---------|
| White blood cell (10^9/L)       | 7.1±2.1      | 6.9±2.1       | 0.514   |
| Red blood cell (10^{12}/L)      | 4.6±0.6      | 4.6±0.6       | 0.773   |
| Platelet (10^9/L)               | 210.8±92.6   | 221.7±55.7    | 0.290   |
| Total cholesterol (mmol/L)      | 4.4±0.9      | 4.3±0.8       | 0.259   |
| Low-density lipoprotein (mmol/L)| 2.7±0.8      | 2.4±0.8       | 0.003*  |
| High-density lipoprotein (mmol/L)| 1.1±0.3     | 1.1±0.3       | 0.103   |
| Uric acid(μmol/L)               | 329.8±87.9   | 330.3±95.9    | 0.972   |
| Homocysteine(μmol/L)            | 18.9±10.5    | 18±12.3       | 0.610   |
| Fasting blood glucose (mmol/L)  | 5.9±1.8      | 5.6±1.3       | 0.231   |
| Hemoglobin A1c (%)              | 6.4±1.4      | 6.3±1.2       | 0.737   |
| Glycated albumin(%)             | 13.8±2.4     | 17.5±3.8      | 0.060   |
| Fibrinogen(mg/dl)               | 268.5±82.2   | 271.8±87.3    | 0.843   |
| C-reactive protein (mg/L)       | 2.05(1.2─4.0)| 2.25(0.89─4.56)| 0.925   |
| 25-hydroxy vitamin D (ng/mL)    | 10.2±5.6     | 14.6±7.5      | 0.001*  |
| Albumin (g/L)                   | 42.1±4.4     | 42.5±4.8      | 0.681   |

Values represent number (percent) for categorical variables and mean ± SD or median (interquartile range) for continuous variables. *P< 0.05 is statistically significant
### Table 2. Multivariate logistic regression as predictors of the occurrence of SVD

| Predictors                  | P    | OR       | 95% CI for OR |
|-----------------------------|------|----------|---------------|
| Sex                         | 0.127| 0.245    | 0.040-1.493   |
| Hyperlipidemia              | 0.862| 0.875    | 0.194-3.947   |
| Prior Stroke                | 0.070| 5.231    | 0.874-31.310  |
| Smoking                     | 0.061| 0.131    | 0.016-1.095   |
| Systolic blood pressure     | 0.696| 1.008    | 0.967-1.052   |
| Diastolic blood pressure    | 0.750| 0.990    | 0.928-1.056   |
| Low-density lipoprotein     | 0.170| 0.585    | 0.272-1.258   |
| 25-hydroxy vitamin D        | 0.001| 0.772    | 0.691-0.862   |

*P< 0.05 is statistically significant

### Table 3. Association of vitamin D with SVD estimated by odds ratio (OR) at 95% confidence interval (CI)

| Vit D (ng/ml) | SVD(106) | Control(115) | Model 1 | Model 2 |
|---------------|----------|--------------|---------|---------|
|              |          |              |         |         |
| ≥20          | 11(10.4%)| 25(21.7%)    | 1(Ref)  | 1(Ref)  |
| 12-20        | 19(17.9%)| 43(37.4%)    | 0.740 [1.171(0.461-2.972)] | 0.9[1.077(0.338-3.428)] |
| <12          | 76(71.7%)| 47(40.9%)    | 0.001*[4.154 (1.797-9.599)] | 0.001*[5.609(2.006-15.]

Model 1 adjusted with age and gender;

Model 2 additionally adjusted with hyperlipidemia, stroke, smoking history, systolic blood pressure, diastolic blood pressure, low-density lipoprotein, diabetes, hypertension.

*P< 0.05 is statistically significant

### Table 4. Odds ratio (OR) at 95% confidence interval (CI) of SVD in hypertensives for three groups of serum 25(OH)D

| Vit D (ng/ml) | Model 1 | Model 2 | P-interactio |
|---------------|---------|---------|--------------|
|               | P-OR (95% CI) | P-OR (95% CI) |   |
| Hypertensive  |         |         | 0.001*       |
| ≥20           | 1(Ref) |         |              |
| 12-20         | 0.980 | 0.985(0.294-3.295) | 0.898 | 1.108(0.232-5.280) |
| <12           | 0.002*| 5.461(1.0-15.651)  | 0.001*| 9.738(2.398-39.540) |
*P < 0.05 is statistically significant