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Original Research Article

ASSOCIATION BETWEEN SERUM HOMOCYSTEINE AND B VITAMINS (VITAMIN B6, B12 AND FOLIC ACID) IN STROKE

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ABSTRACT

Background: Hyperhomocysteinemia has been identified as a potential modifiable risk factor for stroke. Deficiencies of vitamin B6, vitamin B12, and folic acid may impair homocysteine metabolism, leading to elevated serum levels and increased vascular risk. The objective is to evaluate the association between serum homocysteine and B vitamins (vitamin B6, vitamin B12, and folic acid) in patients with stroke and to compare findings between ischemic and hemorrhagic subtypes.

Materials and Methods: This hospital-based cross-sectional study included 70 patients with radiologically confirmed stroke. Serum homocysteine, vitamin B6, vitamin B12, and folic acid levels were measured using standard laboratory methods. Statistical analysis was performed using SPSS version 26, and $p < 0.05$ was considered significant.

Results: The mean age of participants was 59.94 ± 14.099 years, with males comprising 62.9% of cases. Ischemic stroke accounted for 75.7% and hemorrhagic stroke for 24.3% of cases. Mean homocysteine level was 17.59 ± 11.37 µmol/L, and 57.1% of patients had hyperhomocysteinemia. Mean vitamin B6 level was 9.47 ± 12.63 ng/ml, with deficiency observed in 52.9% of patients. Vitamin B6 levels were significantly lower in hemorrhagic stroke (5.70 ng/ml) compared to ischemic stroke (10.67 ng/ml) ($p = 0.017$). Hyperhomocysteinemia was more prevalent in ischemic stroke (62.3%) than hemorrhagic stroke (41.2%) ($p = 0.033$). No significant correlation was observed between homocysteine and B vitamin levels ($p > 0.05$).

Conclusion: Hyperhomocysteinemia and vitamin B6 deficiency were common among stroke patients. Elevated homocysteine was more strongly associated with ischemic stroke, while lower vitamin B6 levels were significantly linked to hemorrhagic stroke. These findings suggest that homocysteine metabolism and B vitamin status may play a role in stroke subtypes and should be considered in clinical evaluation.

Keywords: Stroke, Homocysteine, Vitamin B6, Vitamin B12, Folic Acid, Hyperhomocysteinemia, Ischemic Stroke, Hemorrhagic Stroke.

INTRODUCTION

Stroke is one of the most significant health issues in the world and the second causes of death worldwide, which causes a significant number of people disability in the long term and a significant burden on healthcare.[1] The majority of cases occurred in ischemic stroke, whereas less common but with higher mortality and outcomes are hemorrhagic stroke.[2,3] Regardless of the known risk factors, including hypertension, diabetes mellitus, dyslipidemia, smoking, and atrial fibrillation,[4,5] the incidence of stroke is on the increase, which implies that other metabolic factors are at play. One of the potentially modifiable risk factors of the vascular risk has become hyperhomocysteinemia.[6] Homocysteine is an amino acid of sulfur that is synthesized as a result of methionine metabolism and is regulated by remethylation and transsulfuration processes that involve the cofactors folic acid, vitamin B12 and

vitamin B6 among others.[7] Lack of these B vitamins interferes with the metabolism of homocysteine so that the level of this chemical increases in plasma.[8] High homocysteine enhances endothelial dysfunction, oxidative stress and vascular inflammation, thus facilitating atherosclerosis and predisposing to thrombosis.[9] It also has procoagulant effects and reduction of the bioavailability of nitric oxide, further leading to cerebrovascular injury.[10,11] Epidemiological research has also proved that there is a positive correlation between elevated levels of homocysteine and stroke risk that are independent of conventional factors,[12] and that the relationship has been supported by meta-analyses.[13]

Nonetheless, randomized trials assessing B vitamin supplementation have reported conflicting outcomes as far as stroke prevention is concerned despite successful homocysteine lowering.[14] This has been controversial as to whether homocysteine is a causal or a surrogate vascular pathology marker. MTHFR mutations,[15] as well as nutritional deficiencies and renal dysfunction,[16] are also genetic polymorphisms that complicate this association. There is also possibly an emerging association of hyperhomocysteinemia with hemorrhagic stroke because of the processes of vascular fragility.[17] Considering such complexities, the assessment of serum homocysteine in conjunction with the vitamin B6, vitamin B12 and folic acid of stroke patients would continue to be clinically important. Better knowledge of these biochemical interactions can be used to improve the risk stratification and provide nutritional interventions in stroke management.

MATERIALS AND METHODS

This was a hospital-based cross-sectional study conducted in the Department of General Medicine at Shri B.M. Patil Medical College Hospital and Research Centre, Vijayapura, Karnataka. The study was carried out over a period from March 2024 to December 2025. A total of 70 patients diagnosed with stroke were included in the study. Patients admitted to the hospital with clinical features suggestive of stroke and radiological confirmation were enrolled consecutively.

Inclusion Criteria

- Patients aged ≥18 years.
- Clinically diagnosed cases of stroke with confirmation by computed tomography (CT) or magnetic resonance imaging (MRI) of the brain.
- Both ischemic and hemorrhagic stroke patients.
- Patients who provided informed consent.

Exclusion Criteria

- Patients with transient ischemic attack (TIA).
- Patients with known renal failure or hepatic failure.
- Patients receiving vitamin B supplementation prior to admission.
- Patients with known hematological disorders or malignancy.
- Patients unwilling to participate.

Data Collection

After obtaining written informed consent, detailed demographic and clinical information was collected from all enrolled patients using a structured proforma. Data included age, gender, presenting complaints, duration of symptoms, and relevant vascular risk factors such as hypertension, diabetes mellitus, smoking, alcohol consumption, and dyslipidemia. The type of stroke was determined based on clinical evaluation and confirmed through neuroimaging (CT or MRI brain), and patients were classified as having ischemic or hemorrhagic stroke accordingly. Under aseptic precautions, venous blood samples were obtained from each patient for estimation of serum homocysteine, vitamin B6, vitamin B12, and folic acid levels. All biochemical analyses were performed in the institutional laboratory using standard laboratory procedures and reference ranges. The collected data were systematically recorded and verified prior to statistical analysis.

Outcome Measures

The primary objective was to evaluate the association between serum homocysteine levels and B vitamin levels (vitamin B6, vitamin B12, and folic acid) in stroke patients. Secondary analysis included comparison of these biochemical parameters between ischemic and hemorrhagic stroke subtypes.

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using SPSS software version 26. Continuous variables were expressed as mean ± standard deviation (SD), and categorical variables were presented as frequency and percentage. Comparisons between groups were performed using appropriate statistical tests. Correlation analysis was conducted to assess the relationship between homocysteine and B vitamin levels. A p-value of <0.05 was considered statistically significant.

Ethical Considerations

The study was conducted after obtaining approval from the Institutional Ethics Committee. Written informed consent was obtained from all participants prior to enrollment.

RESULTS

A total of 70 patients were included in the study, with a mean age of 59.94 ± 14.099 years, indicating a predominantly middle-to-elderly population with moderate age variability. Males constituted the majority of cases, accounting for 44 patients (62.9%), while females comprised 26 patients (37.1%). Ischemic stroke was the dominant subtype, observed in 53 patients (75.7%), whereas hemorrhagic stroke was seen in 17 patients (24.3%), reflecting the typical epidemiological distribution of stroke types.

Table 1: Demographic and Stroke Type Distribution (n = 70)

Variable	Category	Value
Total Patients	Study Population	70 (100%)
Age	Mean ± SD	59.94 ± 14.099
Gender	Male	44 (62.9%)
Gender	Female	26 (37.1%)
Stroke Type	Ischemic	53 (75.7%)
Stroke Type	Hemorrhagic	17 (24.3%)

The overall biochemical profile demonstrated a mean vitamin B6 level of 9.47 ± 12.63 ng/ml, showing wide variability within the population. Vitamin B12 levels were comparatively high, with a mean of 962.21 ± 787.98 pg/ml, also displaying substantial dispersion. Folic acid levels averaged 6.38 ± 5.65 ng/ml. Mean serum homocysteine was elevated at 17.59 ± 11.37 µmol/L, exceeding the defined threshold for hyperhomocysteinemia in a considerable proportion of patients.

Table 2: Overall Biochemical Profile (n = 70)

Parameter	Mean ± SD	Interpretation
Vitamin B6 (ng/ml)	9.47 ± 12.63	37 (52.9%) Low
Vitamin B12 (pg/ml)	962.21 ± 787.98	70 (100%) Assessed
Folic Acid (ng/ml)	6.38 ± 5.65	70 (100%) Assessed
Homocysteine (µmol/L)	17.59 ± 11.37	40 (57.1%) Elevated

Hyperhomocysteinemia (>14.8 µmol/L) was present in 40 patients (57.1%), indicating that more than half of the stroke cohort had elevated homocysteine levels, while 30 patients (42.9%) had normal levels. Vitamin B6 deficiency was observed in 37 patients (52.9%), whereas 33 patients (47.1%) had normal vitamin B6 levels. Among stroke subtypes, elevated homocysteine was more frequent in ischemic stroke patients, affecting 33 of 53 cases (62.3%), compared to 7 of 17 hemorrhagic stroke cases (41.2%).

Table 3: Biochemical Abnormalities (n = 70)

Variable	Category	Value
Hyperhomocysteinemia (>14.8 µmol/L)	Present	40 (57.1%)
Hyperhomocysteinemia (>14.8 µmol/L)	Absent	30 (42.9%)
Vitamin B6 Status	Low	37 (52.9%)
Vitamin B6 Status	Normal	33 (47.1%)
Stroke Type with Elevated Homocysteine	Ischemic	33 (62.3%)
Stroke Type with Elevated Homocysteine	Hemorrhagic	7 (41.2%)

Comparison between stroke subtypes revealed that mean vitamin B6 levels were significantly lower in hemorrhagic stroke patients (5.70 ng/ml) compared to ischemic stroke patients (10.67 ng/ml), with statistical significance ($p = 0.017$). Hyperhomocysteinemia was more prevalent in ischemic stroke (33 patients, 62.3%) than in hemorrhagic stroke (7 patients, 41.2%), and this difference was also statistically significant ($p = 0.033$).

Table 4: Comparison of Key Parameters by Stroke Type

Parameter	Ischemic Stroke (n=53)	Hemorrhagic Stroke (n=17)	p-value
Vitamin B6 (Mean ng/ml)	10.67	5.70	0.017
Hyperhomocysteinemia	33 (62.3%)	7 (41.2%)	0.033
Group Proportion	53 (75.7%)	17 (24.3%)	0.033

DISCUSSION

The current research showed that hyperhomocysteinemia was present in over 57.1% of the stroke patients and the value of homocysteine was 17.59 +11.37 µmol/L. This observation supports the ever-increasing evidence that high levels of homocysteine are typical of people with cerebrovascular disease. Similar findings have been reported in previous studies, in which elevated homocysteine levels were significantly associated with the incidence and severity of stroke, while vascular risk profiling has also been shown to be important in other major cardiovascular conditions.[18] The hypothesis that hyperhomocysteinemia can be causing factors in the pathogenesis of stroke, including the vascular endothelial dysfunction, oxidative stress and prothrombotic conditions, is supported by the high mean value found in this cohort.[19] Mean level was found to be 9.47 ± 12.63 ng/ml, which indicated that there was a lot of variation in the population, yet there was the identification of 52.9% of deficiency of vitamin B6. Interestingly, the mean level of vitamin B6 was much lower in hemorrhagic stroke patients (5.70 ng/ml) than in the ischemic stroke patients (10.67 ng/ml), which also was statistically significant ($p = 0.017$). Past studies have also indicated that levels of vitamin B6 are lower in stroke patients and some studies have indicated that there is a greater correlation between vitamin B6 deficiency and

hemorrhage probably because of its involvement in vascular integrity as well as in the dampening of inflammation. The results of these investigations imply that the effect of vitamin B6 deficiency on the subtypes of stroke can vary.[20] There was a statistically significant difference between hyperhomocysteinemia in patients with ischemic stroke (62.3) and hemorrhagic stroke (41.2). This is consistent with earlier studies that reveal that high levels of homocysteine are more associated with ischemic stroke especially relative to atherosclerosis and thrombotic processes. The more common prevalence of homocysteinemia in ischemic stroke in this study favors the idea that homocysteine is not a significant factor in the hemorrhagic but rather the ischemic cerebrovascular pathology.[21]

Even though the level of vitamin B12 (mean 962.21 ± 787.98 pg/ml) and folic acid (mean 6.38 ± 5.65 ng/ml) were measured in all patients, no significant relationship between homocysteine and either of the B vitamins was found ($p > 0.05$). Such results are not new in the previous studies, as homocysteine levels have not always been correlated with personal concentrations of B vitamins even though their correlation would be plausible biologically. The difference can be attributed to multifactorial effects of genetic polymorphisms, kidney performance, food habits and fluctuation in metabolism.[22] In general, the results of this research confirm the existence of high levels of homocysteine and vitamin B6 deficiency among stroke patients, and the differences are determined by subtypes of ischemic and hemorrhagic stroke. As with the existing literature, it is indicated that the findings of the research might indicate that distortions in homocysteine metabolism and the state of the B vitamins can be associated with the risk of a stroke, yet the causal interdependence is multifactorial.

CONCLUSION

It is concluded that hyperhomocysteinemia and vitamin B6 deficiency were highly prevalent among stroke patients in this study. Elevated homocysteine levels were more frequently observed in ischemic stroke, while significantly lower vitamin B6 levels were associated with hemorrhagic stroke. Although no statistically significant correlation was found between homocysteine and B vitamin levels, the high burden of biochemical abnormalities suggests a potential role of homocysteine metabolism and vitamin status in stroke pathophysiology. These findings highlight the importance of routine assessment of homocysteine and B vitamin levels in stroke patients and warrant further large-scale studies to clarify their clinical significance in stroke prevention and management.

REFERENCES

1. Welch GN, Loscalzo J. Homocysteine and atherothrombosis. N Engl J Med Apr. 1998;9(15):1042–50. https://doi.org/10.1056/nejm199804093381507.
2. Wu DF, Yin RX, Deng JL. Homocysteine, hyperhomocysteinemia, and H-type hypertension. Eur J Prev Cardiol Jul. 2024;23(9):1092–103. https://doi.org/10.1093/eurjpc/zwae022.
3. Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. Jama Oct. 1995;4(13):1049–57. https://doi.org/10.1001/jama.1995.03530130055028.
4. Toole JF, Malinow MR, Chambless LE, et al. Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death: the vitamin intervention for stroke prevention (VISP) randomized controlled trial. Jama Feb. 2004;4(5):565–75. https://doi.org/10.1001/jama.291.5.565.
5. Smith AD, Refsum H. Homocysteine - from disease biomarker to disease prevention. J Intern Med Oct. 2021;290(4):826–54. https://doi.org/10.1111/joim.13279.
6. Ganguly P, Alam SF. Role of homocysteine in the development of cardiovascular disease. Nutr J Jan. 2015;10:14:6. https://doi.org/10.1186/1475-2891-14-6.
7. Huang X, Li Y, Li P, et al. Association between percent decline in serum total homocysteine and risk of first stroke. Neurology Nov. 2017;14(20):2101–7. https://doi.org/10.1212/wnl.0000000000004648.
8. Center For Cardiovascular Diseases The Writing Committee Of The Report On, Cardiovascular H. Diseases in China N. Report on cardiovascular health and diseases in China 2023: an updated summary. Biomed Environ Sci Sep. 2024;20(9):949–92. https://doi.org/10.3967/bes2024.162.
9. Luo Y, Xia F, Yu X, Li P, Huang W, Zhang W. Long-term trends and regional variations of hypertension incidence in china: a prospective cohort study from the China health and nutrition Survey, 1991–2015. BMJ Open Jan. 2021;13(1):e042053. https://doi.org/10.1136/bmjopen-2020-042053.
10. Wang Z, Chen Z, Zhang L, et al. Status of hypertension in china: results from the China hypertension Survey, 2012–2015. Circulation May. 2018;29(22):2344–56. https://doi.org/10.1161/circulationaha.117.032380.
11. Li J, Mao C, Xu B, et al. China precision nutrition and Health-KAP (Knowledge, Attitude, and Practice) Real-World study (CPNAS): enrollment progress and baseline characteristics. Precision Nutr. 2025;4(1):e00095. https://doi.org/10.1097/pn9.0000000000000095.
12. Bates CJ, Vitamin A. Lancet. 1995;345(8941):31 – 5.https://doi.org/10.1016/s0140-6736(95)91157-x
13. Song P, Wang J, Wei W, Chang X, Wang M, An L. The prevalence of vitamin A deficiency in Chinese children: A systematic review and Bayesian meta-analysis. Nutrients. 2017;9(12):1285. https://doi.org/10.3390/nu9121285.
14. Mawson AR, Onor GI. Gout and vitamin A intoxication: is there a connection? Semin Arthritis Rheum Apr. 1991;20(5):297–304. https://doi.org/10.1016/0049-0172(91)90030-4.
15. Moll S, Varga EA. Homocysteine and MTHFR mutations. Circulation. 2015;132(1):e6–e9. https://doi.org/10.1161/CIRCULATIONAHA.114.013311.
16. Jakubowski H. Homocysteine modification in protein Structure/Function and human disease. Physiol Rev Jan. 2019;1(1):555–604. https://doi.org/10.1152/physrev.00003.2018.
17. Xu BP, Shi H. Precision nutrition: concept, evolution, and future vision. Precis Nutr. 2022;1(1):e00002. https://doi.org/10.1097/PN9.0000000000000002.
18. Ahmad MS, Alharbi AOM, Tawakul A, Alturiqy AM, Alzahrani M, Shaik RA, et al. A case-control study on risk factors and outcomes in congestive heart failure. Rev Cardiovasc Med. 2025;26(3):26601.
19. Yun S, Ping C, ZP A, Jianping L, Hanping S. Precision nutrition: 8 stages and 5 dimensions. Precision Nutr.

2023;2(4):e00057.
https://doi.org/10.1097/pn9.0000000000000057.
20. Tianyu C, Xiao H, Ping C, et al. Distribution and status of vitamin B12 in Chinese adults with hypertension: a comprehensive report across 14 provinces. Precision Nutr. 2023;2(4):e00060.
https://doi.org/10.1097/pn9.0000000000000060.
21. Xie H, Zhang K, Wei Y, Ruan G, Zhang H, Li S, Song Y, Chen P, Liu L, Wang B, Shi H. The association of serum betaine concentrations with the risk of new-onset cancers: results from two independent nested case-control studies. Nutr Metab (Lond). 2023;20(1):46.
https://doi.org/10.1186/s12986-023-00755-y.
22. Huo Y, Li J, Qin X, et al. Efficacy of folic acid therapy in primary prevention of stroke among adults with hypertension in china: the CSPPT randomized clinical trial. Jama Apr. 2015;7(13):1325–35.
https://doi.org/10.1001/jama.2015.2274.