Evaluation of Homocysteine Level as a Risk Factor among Patients with Ischemic Stroke and Its Subtypes

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Introduction

Stroke is a heterogeneous condition and its subtypes have different pathophysiological mechanisms and etiologies. Despite a gradual decline in overall stroke death rates in many industrialized countries, stroke remains a leading cause of death and disability in the world.1 Ischemic stroke can be caused by large artery atherosclerotic disease, small vessel or penetrating artery disease...
Homocysteine (Hcy) is a four-carbon amino acid with a free thiol group, which is formed by demethylation of methionine, an essential amino acid derived from diet. Normal total Hcy (tHcy) concentrations range from 5-15 µmol/L in the fasting state. Hyperhomocysteinemia (HHcy) has been classified into moderate (plasma tHcy concentrations of 15-30 µmol/L), intermediate (plasma tHcy concentrations of 31-100 µmol/L), and severe (plasma tHcy concentrations >100 µmol/L). Both acquired and genetic factors can have an impact on plasma tHcy. Male gender, aging, smoking, impaired renal function, and some medications such as Corticosteroids and Cyclosporine are some examples of the acquired causes and classic homocytinuria and C677T homozygote mutation of 5,10-methylenetetrahydrofolate reductase (MTHFR) are the main genetic ones. Vitamin B12, vitamin B6, and folate, all of which have dietary origins, are three main cofactors in Hcy metabolism. Deficiencies in these supplements are more prevalent in the most developing countries and may account for many cases of moderate hyperhomocysteinemia and increased risk of stroke. Tan et al. conducted a study in 2,471 Chinese men and women and showed that decreased plasma levels of folate, vitamin B12, and vitamin B6 as well as male gender and living in urban areas were significantly related to hyperhomocysteinemia.

Several studies have postulated that elevated tHcy is a strong and independent risk factor for vascular diseases including ischemic cerebral stroke. Tan et al. studied 109 young adult Asians (Chinese, Indians, and Malays) with ischemic stroke and found a strong relationship between increased Hcy and ischemic stroke (OR=5.17, 95% CI: 1.96 to 13.63; P=0.001). Other studies have reported the same results in Turkish and Malay populations with ischemic stroke. Furthermore, Biswas et al. conducted a study in 120 Indian patients with acute ischemic stroke and showed that there was a significant relationship between HHcy and ischemic stroke (P=0.001). They also found decreased serum concentrations of vitamin B12 and folate in a significant number of their patients and the role of MTHFR 677 C>T polymorphisms in hyperhomocysteinemia in some of their patients.

Oxidative damage to the vascular endothelium and the proliferation of the vascular smooth muscle create a prothrombotic condition, which contributes to the development of premature atherosclerosis. Moreover, HHcy has been found as a potential risk factor for cardiovascular disease and vascular dementia. Some studies have shown that even mildly increased plasma tHcy can also be a significant risk factor for stroke, more specifically ischemic stroke. The aim of this study was to evaluate HHcy as a risk factor for ischemic stroke and its relationship to specific subgroups of stroke in an Iranian population.

Patients and Methods

Patients and Controls

From January 2009 to January 2010, this case-control study was conducted in 171 patients aged over 16 years within 5 days of their first ischemic stroke in Nemazee Hospital, affiliated to Shiraz University of Medical Sciences. Each case was evaluated by brain computed tomography (CT) within 24 hours of admission and by duplex ultrasound of extracranial vessels and echocardiography (transthoracic or transesophageal) within the next 3 post-stroke days. Brain magnetic resonance imaging (MRI) with MR angiography was performed in some cases. Controls included 86 age and sex-matched persons without ischemic stroke, who visited our Pathobiology Laboratory for blood sampling. Baseline demographic data (age and sex) and conventional cardiovascular risk factors, including diabetes mellitus (DM), hypertension (HTN), hyperlipidemia (HLP), smoking, and previous coronary diseases, were recorded for the patients and controls. All the patients and controls gave their written informed consent, and the Medical Research Ethics Committee of Shiraz University of Medical Sciences approved the study (approval number: 2817).

Sample Collection

Fasting blood samples were obtained from all the patients within 5 days of ischemic stroke and were immediately chilled on ice. Serum samples were collected within 30 minutes and were thereafter stored at -80°C. The axis homocysteine enzyme immunoassay (EIA) (Axis-Shield Diagnostics Ltd., United Kingdom) was used for the quantitative analysis of total L-homocysteine in serum. Vitamin B12 and folate were measured using the SimulTRAC-SNB Radioassay Kit (DRG Instruments GmbH, Germany).

Exclusion Criteria for Cases and Controls

The exclusion criteria were concomitant history of previous ischemic strokes, cerebral venous thrombosis, cardiogenic embolism, nonatherosclerotic vasculopathies, hypercoagulable disorders, or infarcts of undetermined causes. Ischemic strokes account for approximately 80% to 88% of all strokes. The most recognized mechanistic classification is the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification. Homocysteine (Hcy) is a four-carbon amino acid with a free thiol group, which is formed by demethylation of methionine, an essential amino acid derived from diet. Normal total Hcy (tHcy) concentrations range from 5-15 µmol/L in the fasting state. Hyperhomocysteinemia (HHcy) has been classified into moderate (plasma tHcy concentrations of 15-30 µmol/L), intermediate (plasma tHcy concentrations of 31-100 µmol/L), and severe (plasma tHcy concentrations >100 µmol/L). Both acquired and genetic factors can have an impact on plasma tHcy. Male gender, aging, smoking, impaired renal function, and some medications such as Corticosteroids and Cyclosporine are some examples of the acquired causes and classic homocytinuria and C677T homozygote mutation of 5,10-methylenetetrahydrofolate reductase (MTHFR) are the main genetic ones. Vitamin B12, vitamin B6, and folate, all of which have dietary origins, are three main cofactors in Hcy metabolism. Deficiencies in these supplements are more prevalent in the most developing countries and may account for many cases of moderate hyperhomocysteinemia and increased risk of stroke. Tan et al. conducted a study in 2,471 Chinese men and women and showed that decreased plasma levels of folate, vitamin B12, and vitamin B6 as well as male gender and living in urban areas were significantly related to hyperhomocysteinemia.

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Exclusion Criteria for Cases and Controls

The exclusion criteria were concomitant history of previous ischemic strokes, cerebral venous
infarcts, ischemic heart disease, peripheral vascular disease, hypothyroidism, epilepsy, renal impairment, pregnancy, postpartum state, consumption of oral contraceptives or drugs that might affect serum vitamin B12, folate, and Hcy levels, brain mass or any malignancy, history of migraine, and vitamin B12 and folate deficiencies.

**Stroke Subtypes**

According to the TOAST criteria, stroke subtypes were classified into large artery, cardioembolic, small artery/lacunar strokes, and strokes of other undetermined etiologies. All the patients were subtyped using a modified TOAST criterion. Patients with incomplete data because of early death or other causes were gathered in the last group.

**Statistical Analysis**

All the analyses were done using SPSS (version 13) software (SPSS, Inc.). Student’s t test was used for the quantitative variables. Chi-square test was used to analyze the qualitative findings. Odds ratios (OR) and 95% confidence intervals were calculated and a P value smaller than 0.05 was considered statistically significant. Age, sex, DM, and smoking were matched in both cases and controls (table 1). HTN and HLP were controlled by stratifying two levels. Binary logistic regression analysis was used to calculate Hcy in the stroke subgroups and controls. Additionally, the analysis of variance (ANOVA) was employed to compare the mean values of B12, folate, and Hcy.

**Results**

One hundred seventy-one consecutive patients and 86 age and sex-matched controls from the same geographic area were selected. Table 1 shows the baseline demographic values, conventional vascular risk factors, fasting serum Hcy, vitamin B12, and folate levels in the cases and controls. Table 2 illustrates fasting serum Hcy, vitamin B12, and folate levels in the stroke subtypes and controls.

In this study, our findings showed that mean levels of fasting serum Hcy were significantly higher in the cases than in the controls (16.2 μmol/L vs. 13.5 μmol/L).
Fasting serum homocysteine, vitamin B12, and folate levels in stroke subtypes and controls

| Stroke subtype       | Large-vessel (n=24) | Cardio-embolic (n=56) | P* | Small-vessel/Lacunar (n=33) | P* | Undetermined causes (n=32) | P* | Other causes (n=26) | P* | Controls (n=86) |
|----------------------|---------------------|-----------------------|----|---------------------------|----|---------------------------|----|-------------------|----|-----------------|
| Mean Hcy level (μmol/L) (95% CI) | 13.7 (10.6-16.8) | 17.7 (14.8-20.5) | 0.010 | 14.2 (11.7-16.6) | 0.988 | 16.4 (13.3-19.5) | 0.081 | 17.4 (13.5-21.3) | 0.029 | 13.5 (12.4-14.6) |
| Mean B12 level (pmol/L) (95% CI) | 225.7 (163.8-287.6) | 0.576 | 387.9 (297.4-478.3) | 1.000 | 332.3 (239.3-425.3) | 0.753 | 289.5 (207.3-371.7) | 0.355 | 330.9 (227.9-433.9) | 0.569 | 386.1 (333.4-436.8) |
| Mean folate level (nmol/L) (95% CI) | 5.7 (4.9-6.5) | 0.390 | 7.0 (6.2-7.8) | 0.735 | 6.4 (5.6-7.2) | 0.993 | 6.2 (5.3-7.0) | 0.874 | 6.9 (5.7-8.0) | 0.733 | 6.56 (6.1-7.1) |

*Dunette-Test, comparing each group with the controls separately

13.5 μmol/L; P=0.013). The mean Hcy level was significantly higher in the cardioembolic group than in the controls after adjustment for HTN and HLP (17.7 μmol/L vs. 13.5 μmol/L; P=0.008). No other stroke subtypes showed significantly different Hcy levels after adjustment compared with the controls. There was a significant difference in vitamin B12 level between the large vessel subgroup and the controls before adjustment for HTN and HLP (P=0.033), but the difference was not significant after adjustment. Also, the difference in folate level between the cases and controls was not statistically significant (6.52 nmol/L vs. 6.56 nmol/L; P=0.908). Our study showed that fasting Hcy had a strong, graded, and independent relationship with the risk of ischemic stroke. The odds ratio of 2.17 (95% CI: 1.24 to 3.79; P=0.004) for Hcy above 15 μmol/L concentration for all types of stroke was achieved. Fasting Hcy was also a strong risk factor for the cardioembolic subtype (OR=2.8, 95% CI:1.4 to 5.6; P=0.05) for Hcy above 15 μmol/L in our patients, but not for the large vessel or lacunar or the other undetermined categories.

Discussion

Over the last decade, convincing evidence has been gathered on the relation between moderate elevation of plasma tHcy and ischemic stroke. Several studies have reported that HHcy is associated with two to threefold increased risk of ischemic stroke. In 1995, Boushey et al. reported the results of the first meta-analysis of 27 observational studies on Hcy and atherosclerotic vascular disease, of which 11 studies addressed the association between Hcy and risk of stroke. Nine case-control studies provided support for the hypothesis that Hcy is an independent risk factor for stroke, while 2 prospective studies reported negative results. Similar to our findings, the odds ratio of this meta-analysis for cerebrovascular disease in patients with elevated Hcy levels was 2.5 (95% CI, 2.0 to 3.0). In 6 studies with fasting blood samples, the odds ratio for a 5 μmol/L increment in Hcy showed that there was an approximately twofold increase in risk (OR=1.9; 95% CI, 1.6 to 2.3). Similar to our findings, several Asian studies have shown the independent role of HHcy in increasing the risk of ischemic strokes. However, some of these studies have had the confounding effects of nutritional deficiencies (such as vitamin B12, vitamin B6, and folate). Omrani et al. conducted a study in 93 Iranian patients with acute ischemic stroke and concluded that HHcy was a risk factor for ischemic stroke. They did not study the relationship between HHcy and ischemic stroke subtypes, but showed that there was a significant relationship between HHcy and smoking in their patients group.

Studies which have evaluated the relation between Hcy levels and stroke subtypes have shown different results. A Swedish study in 57 stroke patients with HHcy reported significantly higher tHcy in all stroke subtypes. Eikelboom et al. reported that tHcy was significantly greater in large artery and small vessel stroke compared with cardioembolic and controls. Tan et al. showed that increased t-Hcy was associated with a higher risk of large artery stroke. Two other studies in a Turkish population demonstrated that HHcy had a significant role in lacunar and large vessel atherothrombotic, increased intimal media thickness of extracranial carotid arteries, and severe carotid stenosis. Other studies have shown a relation between increased t-Hcy and lacunar stroke and carotid stenosis.

Our findings on the relationship between HHcy and cardioembolic subgroup may be explained by higher prevalence of cardiac disease in our country or the fact that our center is a referral center and most uncomplicated patients that have fewer vascular risk factors are not referred to this center. These findings may support the hypothesis that HHcy has different mechanisms of pathogenicity, which may show the influence of other undiagnosed genetic and environmental factors acting as confounders.

Several factors contribute to increased
Homocysteine levels in ischemic stroke and its subtypes

plasma Hcy levels. Individuals with pre-existing atherosclerosis have higher Hcy levels than those without pre-existing atherosclerosis. It seems that there is an association between economic prosperity and the risk of stroke. Higher prevalence of HHcy in many developing countries could indicate the role of inadequate intake of vitamins and antioxidants in the multi-factorial causes of stroke. The effect of genetic factors on hyperhomocysteinemia is also important. In fact, these factors may confound the results of epidemiological studies and may render the results statistically unstable.

This study has some important limitations. First, intracranial atherosclerosis can give rise to lacunar infarcts indistinguishable from lacunes and may result in small vessel/lacunar misclassification. Furthermore, small cardioembolic emboli can cause lacunar syndromes, acting as a confounding factor in the analysis of the relation between HHcy and stroke subtypes. We also could not omit HHcy as an acute-phase reactant and possible genetic propensity of our patients to HHcy.

In our study, we tried to match all the previously known traditional risk factors of cerebrovascular disease in the selection of controls. However, it was achieved only for age, sex, DM, and smoking and we resolved the confounding actions of HTN and HLP with statistical methods.

It is deserving of note that no randomized trial has so far shown that lowering tHcy reduces the prevalence of cerebral ischemic events. Boushey et al. demonstrated that the administration of folate, vitamin B12, and vitamin B6 decreased Hcy. Furthermore, Biswas et al. reported that taking 5 mg folate decreased Hcy significantly. However, large randomized trials are needed to determine whether decreased tHcy levels by multivitamin therapy can reduce the risk of cardiovascular disease. If a combination of vitamins is found to be effective, this safe, inexpensive, easily administered therapy will probably be widely used throughout the world and have a major effect on public health.

Conclusion

This study showed that an elevated Hcy level was an independent risk factor for ischemic stroke in patients who live in the Iranian province of Fars. In addition, there was a significant relationship between increased Hcy levels and the risk of cardioembolic strokes.

Acknowledgement

The present article was extracted from the thesis written by Dr. M. Fathi in Neurology and was supported by Shiraz University of Medical Sciences (grant number: 2817). The authors would like to thank the Center for Development of Clinical Studies of Nemazee Hospital for statistical assistance and Ms. Hosseini and Ms. Gholami of Shiraz Neurosciences Research Center for their help.

Conflict of interest: None declared.

References

1 Biller J, Love BB, Schneck MJ. Vascular Diseases of the Nervous System. In: Bradley WG, Daroff RB, editors. Neurology in Clinical Practice. 5th ed. Philadelphia: Butterworth-Heinemann; 2008. p. 1165.
2 Goldstein LB, Jones MR, Matchar DB, Edwards LJ, Hoff J, Chilukuri V, et al. Improving the reliability of stroke subgroup classification using the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) criteria. Stroke. 2001;32:1091-8. doi: 10.1161/01.STR.32.5.1091. PubMed PMID: 11340215.
3 Christopher R, Nagaraja D, Shankar SK. Homocysteine and cerebral stroke in developing countries. Curr Med Chem. 2007;14:2393-401. doi: 10.2174/092986707781745613. PubMed PMID: 17896987.
4 Dierkes J, Westphal S. Effect of drugs on homocysteine concentrations. Semin Vasc Med. 2005;5:124-39. doi: 10.1055/s-2005-872398. PubMed PMID: 16047265.
5 Madonna P, de Stefano V, Coppola A, Cirillo F, Cerbone AM, Orefice G, et al. Hyperhomocysteinemia and other inherited prothrombotic conditions in young adults with a history of ischemic stroke. Stroke. 2002;33:51-6. doi: 10.1161/his0102.100483. PubMed PMID: 11779888.
6 Kluijtmans LA, Young IS, Boreham CA, Murray L, McMaster D, McNulty H, et al. Genetic and nutritional factors contributing to hyperhomocysteinemia in young adults. Blood. 2003;101:2483-8. doi: 10.1182/blood.V101.7.2483. PubMed PMID: 12642343.
7 Sánchez-Moreno C, Jiménez-Escrig A, Martín A. Stroke: roles of B vitamins, homocysteine and antioxidants. Nutr Res Rev. 2009;22:49-67. doi: 10.1017/S0955442209990023. PubMed PMID: 19555518.
8 Hao L, Ma J, Zhu J, Stampfer MJ, Tian Y, Willett WC, et al. High prevalence of hyperhomocysteinemia in Chinese adults is associated with low folate, vitamin B-12, and vitamin B-6 status. J Nutr. 2007;137:407-13. PubMed PMID: 17237319.
9 Yokote H, Shiraishi A, Shintani S, Shiigai
T. Acute multiple brain infarction in large-artery atherosclerosis is associated with hyperhomocyst(e)inemia. Acta Neurol Scand. 2007;116:243-7. doi: 10.1111/j.1600-0404.2007.00873.x. PubMed PMID: 17824903.

10 Furie KL, Kelly PJ. Homocyst(e)ine and stroke. Semin Neurol. 2006;26:24-32. doi: 10.1055/s-2006-933306. PubMed PMID: 16479441.

11 Pezzini A, Del Zotto E, Padovani A. Homocysteine and cerebral ischemia: pathogenic and therapeutic implications. Curr Med Chem. 2007;14:249-63. doi: 10.2174/092986707779941140. PubMed PMID: 18757289.

12 Khan U, Crossley C, Kalra L, Rudd A, Wolfe CD, Collinson P, et al. Homocysteine and its relationship to stroke subtypes in a UK black population: the south London ethnicity and stroke study. Stroke. 2008;39:2943-9. doi: 10.1161/STROKEAHA.107.513416. PubMed PMID: 18757289.

13 Tan NC, Venketasubramanian N, Saw SM, Tjia HT. Hyperhomocyst(e)inemia and risk of ischemic stroke among young Asian adults. Stroke. 2002;33:1956-62. doi: 10.1161/01.STR.0000021899.08659.C8. PubMed PMID: 12150425.

14 Boysen G, Brander T, Christensen H, Gideon R, Truelsen T. Homocysteine and risk of recurrent stroke. Stroke. 2003;34:1258-61. doi: 10.1161/01.STR.0000090178.78624.37. PubMed PMID: 12702838.

15 Fallon UB, Virtamo J, Young I, McMaster D, Ben-Shlomo Y, Wood N, et al. Homocysteine and cerebral infarction in in Finnish male smokers. Stroke. 2003;34:1359-63. doi: 10.1161/01.STR.0000074035.64365.2D. PubMed PMID: 12750538.

16 Zylberstein DE, Skoog I, Björkelund C, Guo X, Hultén B, Andreasson LA, et al. Homocysteine levels and lacunar brain infarcts in elderly women: the prospective population study of women in Gothenburg. J Am Geriatr Soc. 2008;56:1087-91. doi: 10.1111/j.1532-5415.2008.01724.x. PubMed PMID: 18554363.

17 Kavaklı HŞ, Altıntaş ND, Tanrıverdi F. Homocysteine levels in acute ischemic stroke patients. JAEM. 2010;9:169-71. doi: 10.5152/ jaem.2010.007.

18 Tan KS, Lee TC, Tan CT. Hyperhomocysteinemia in patients with acute ischaemic stroke in Malaysia. Neurol J Southeast Asia. 2001;6:113-9.

19 Biswas A, Ranjan R, Meena A, Akhter MS, Yadav BK, Munisamy M, et al. Homocystine levels, polymorphisms and the risk of ischemic stroke in young Asian Indians. J Stroke Cerebrovasc Dis. 2009;18:103-10. doi: 10.1016/j.jstrokecerebrovasdis.2008.09.014. PubMed PMID: 19251185.

20 Polidori MC, Cherubini A, Senin U, Mecocci P. Hyperhomocysteinemia and oxidative stress in ischemic stroke. Stroke. 2001;32:275-8. doi: 10.1161/01.STR.32.1.275. PubMed PMID: 11136951.

21 Eikelboom JW, Lonn E, Genest J Jr, Hankey G, Yusuf S. Homocyst(e)ine and coronary artery disease: a critical review of the epidemiologic evidence. Ann Intern Med. 1999;131:363-75. doi: 10.7326/0003-4819-131-5-19990907-00008. PubMed PMID: 10475890.

22 Moghadasian MH, McManus BM, Frohlich JJ. Homocyst(e)ine and coronary artery disease. Clinical evidence and genetic and metabolic background. Arch Intern Med. 1997;157:2299-308. doi: 10.1001/archinte.1997.00440410025003. PubMed PMID: 9361570.

23 Homocysteine Studies Collaboration. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. JAMA. 2002;288:2015-22. doi: 10.1001/jama.288.16.2015. PubMed PMID: 12387654.

24 Korczyn AD. Homocysteine, stroke, and dementia. Stroke. 2002;33:2343-4. doi: 10.1161/01.STR.0000032551.95449.2A. PubMed PMID: 12364715.

25 Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke. 1993;24:35-41. doi: 10.1161/01.STR.24.1.35. PubMed PMID: 7678184.

26 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. 1995;274:1049-57. doi: 10.1001/jama.1995.03530130055028. PubMed PMID: 7563456.

27 Somay G, Alışkan T, Erenoglu NY. Carotid artery stenosis and homocysteine in ischemic stroke. A case-control study. Journal of Neurological Sciences. 2005;22:394-402.

28 Omrani HQ, Shandiz EE, Qabai M, Chaman R, Fard HA, Qaffarpoor M. Hyperhomocysteinemia, folate and B12 vitamin in Iranian patients with acute ischemic stroke. ARYA Atheroscler. 2011;7:97-101. PubMed PMID: 22577454; PubMed Central PMCID: PMC3347852.

29 Eikelboom JW, Hankey GJ, Anand SS,
Lofthouse E, Staples N, Baker RI. Association between high homocyst(e)ine and ischemic stroke due to large- and small-artery disease but not other etiologic subtypes of ischemic stroke. Stroke. 2000;31:1069-75. doi: 10.1161/01.STR.31.5.1069. PubMed PMID: 10797167.

Sasaki T, Watanabe M, Nagai Y, Hoshi T, Takasawa M, Nukata M, et al. Association of plasma homocysteine concentration with atherosclerotic carotid plaques and lacunar infarction. Stroke. 2002;33:1493-6. doi: 10.1161/01.STR.0000016463.01398.D0. PubMed PMID: 12052980.

Streifler JY, Rosenberg N, Chetrit A, Eskaraev R, Sela BA, Dardik R, et al. Cerebrovascular events in patients with significant stenosis of the carotid artery are associated with hyperhomocysteinemia and platelet antigen-1 (Leu33Pro) polymorphism. Stroke. 2001;32:2753-8. doi: 10.1161/hs1201.099650. PubMed PMID: 11739968.

Wierzbicki AS. Homocysteine and cardiovascular disease: a review of the evidence. Diab Vasc Dis Res. 2007;4:143-50. doi: 10.3132/dvdr.2007.033. PubMed PMID: 17654449.