Plasma Homocysteine and Ischemic Stroke Patients in Thailand

Tasanee Tantirittisak MD*, Thanyachai Sura MD**,
Worachat Moleerergpoom MD***, Suchat Hanchaipiboolkul MD*

** Department of Neurology, Prasat Neurological Institute*
*** Division of Genetic, Department of Medicine, Ramathibodi Hospital, Mahidol University*
**** Cardiology Unit, Police General Hospital*

Background: *Hyperhomocysteinemia was recently found to be a risk factor for stroke; however, the available data from Thailand is scarce.*
Objective: *To study plasma homocysteine levels in ischemic stroke and compare it with age-and sex-matched controls, and to identify the association of plasma homocysteine and subtype of stroke.*
Material and Method: *The authors studied plasma homocysteine levels of ischemic stroke patients with clinical signs and symptoms of stroke as confirmed by CT scan and compared them with control subjects who presented with other diseases and no clinical signs and symptoms of stroke between June 2000- May 2001 in Prasat Neurological institute. Fasting plasma homocysteine was measured by HPLC technique. Abnormal cut off point of plasma homocysteine was identified and associations of plasma homocysteine and stroke were studied by using logistic regression analyses.*
Results: *Two hundred and sixty-eight patients were recruited in the present study (132 controls and 136 ischemic stroke patients). The abnormal cut off point of plasma homocysteine was > 14μmol/L. The authors found statically significant association of abnormal plasma homocysteine and stroke ($p<0.001$) with odds ratio of 4.277 (95%CI 2.551-7.171). After adjusting the confounding factor, the authors found that high homocysteine was significantly associated with ischemic stroke ($p<0.001$) with odd ratio of 3.401 (95%CI 1.954-5.922). In the subgroup analyses of type of stroke and abnormal homocysteine, the authors demonstrated that abnormal homocysteine levels were more pronounced in the large vessel subtype than the small group.*
Conclusion: *Abnormal homocysteine level is an independent risk factor of ischemic stroke and more correlated with large vessel subtype.*

Keywords: *Stroke, Homocysteine*

J Med Assoc Thai 2007; 90 (6): 1183-7
Full text. e-Journal: *http://www.medassocthai.org/journal*

Stroke is a common cause of death in the world. In Thailand, stroke is the first cause of death in females and second cause in males[1]. There are many risk factors associated with stroke, such as hypertension, diabetes, smoking, and other risk factors. Elevated plasma homocysteine levels have been indicated as a risk factor for coronary heart disease[2-4], ischemic stroke[5-7], and peripheral artery disease[8,9]. However, most of these findings were derived from white populations, and whether such findings apply for Asians remains to be determined.

The authors studied the association of stroke and homocysteine levels in patients with ischemic stroke and those individuals of similar age and sex without recognized stroke. The cut off point of abnormal homocysteine was also identified.

Correspondence to : Tantirittisak T, Department of Neurology, Prasat Neurological Institute, Rajvithi Rd, Rajthevi, Bangkok 10400, Thailand. E-mail: ttsanee067@yahoo.co.th

Material and Method

Subjects

Subjects for this investigation were enrolled from 268 patients consecutively admitted to Prasat

Neurological Institute between June 2000 and May 2001. Ischemic stroke patients who presented with clinical sudden neurological deficits were diagnosed in 136 of 268 patients by CT scan that showed evidences of cerebral infarction. The 132 control subjects were patients with other diseases who did not have clinical signs and symptoms of cerebral infarction. The exclusion criteria for the current study were malnutrition, HIV positive, and the use of anticonvulsants, multivitamins, and methotrexate. Additionally, because the implication of atherosclerosis is not clear for cardioembolic infarction and unclassified stroke, patients with these conditions were also excluded. All clinical data such as risk factors, types of ischemic stroke by Oxfordshire Community Stroke Project (OCSP) subtypes and concurrent illness were collected.

Evaluation of plasma homocysteine levels

Blood was drawn from fasting subjects. Venous blood samples were collected in the EDTA tubes, centrifuged within 60 minutes, and stored at -20°C to avoid the false elevation in homocysteine concentration as the result of its release from red blood cells[10]. The homocysteine level determined by high-performance liquid chromatography was considered the sum of the homocysteine, the homocysteine moieties of homocysteine, and the cysteine-homocysteine mixed disulfide levels, regardless of whether they were free or protein bound.

Statistical analysis

An abnormal cut off point of plasma homocysteine was identified by ROC curve. Association of homocysteine with stroke was examined by Chi-square test. Unpaired t-test or Mann Whitney U test were used to compare the different between mean $\pm$ standard deviation (SD). The ability of plasma homocysteine levels to stratify the likelihood for ischemic stroke was examined by logistic regression analyses. A value of $p < 0.05$ was considered statistically significant. SPSS version 11.5 statistical software was used for all analyses.

Results

Two hundred and sixty-eight patients were included in the present study. One hundred and thirty six patients and non-stroke 132 control subjects consented to participate. The patients and control groups did not differ significantly in mean age: 61.0 versus 61.5 years. Demographic data for patients and control subjects are shown in Table 1.

The authors identified the abnormal homocysteine level by using ROC curve (cut point at 1-specificity 0.310 and sensitivity 0.654) (Fig. 1) and found that it may be more than 14 μmole/L. Analyses of association between abnormal homocysteine level and ischemic stroke was done by using Pearson Chi-square test and found that abnormal homocysteine level is significantly associated with ischemic stroke ($p < 0.000$) and had odds ratio of 4.277 (95% confidence interval 2.551-7.171). As shown by demographic data, sex and many risk factors (hypertension, diabetes, and smoking) had statistical differences between the patients and control group. Then the authors analyzed the association of abnormal homocysteine level and stroke by adjusting the confounder factors. Using logistic regression analysis, it revealed that this association was also statistically significant ($p < 0.000$) and had an odd ratio of 3.401 (95% confidence interval 1.954-5.922). The correlation of subtypes of stroke and abnormal

Table 1. Demographic data between patients and control subjects

	Stroke (n = 136)	Control (n = 132)	p-value
Mean age (range) (yr)	61.0 (26-85)	61.5 (26-91)	NS$^{\psi}$
Male:female	1:0.7	1:1.19	<0.05$^{\psi}$
Hypertension	66.7%	50.0%	<0.05$^{\psi}$
Diabetes	28.1%	15.9%	<0.05$^{\psi}$
Smoking	28.1%	9.5%	<0.001$^{\psi}$
High LDL*	61.6%	51.4%	NS$^{\psi}$
Ischemic heart disease	20.0%	28.2%	NS$^{\psi}$
Carotid bruit	3.0%	0.0%	NS$^{\psi}$
Mean homocysteine (μmole/L)	16.46 $\pm$ 6.43	12.9 $\pm$ 7.75	<0.001$^{\tau}$

*defined by NCEP III guideline

$^{\psi}$Chi-square test, $^{\tau}$ Mann-Whitney U test

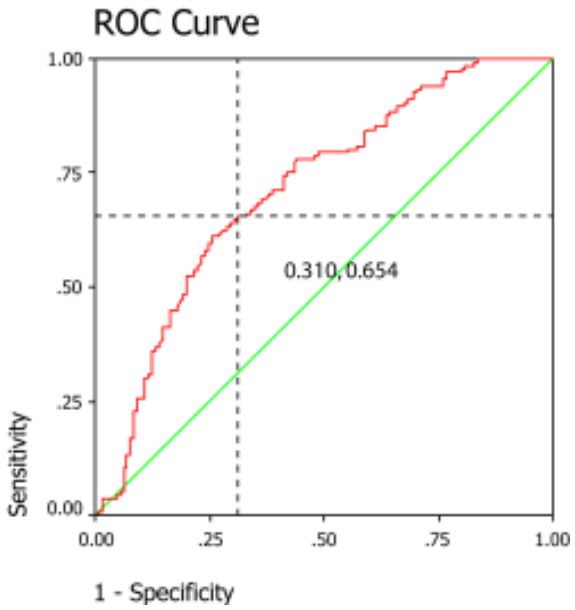

Fig. 1 ROC curve

homocysteine level were evaluated and found that large vessel strokes were statistically significant associated with abnormal homocysteine level ($p < 0.05$) by Pearson Chi-square test.

Discussion

In the present study, the authors found the cut off value of abnormal homocysteine level was > 14 μmole/L. This value is quite similar to that quoted in other studies[11]. In one Thai study in children they defined the abnormal level was above 11.5 μmole/L (the 95th percentile), which is not the same as the presented data. This may be the difference between adults and children[12].

From the presented case-control study, the authors found a strong association of abnormal homocysteine level with ischemic stroke in both analyses before and after adjusting the confounding factors with the odd ratio of 4.277 and 3.401 respectively. This confirmed that hyperhomocysteinemia is a risk factor of ischemic stroke.

The authors also demonstrated that hyperhomocysteine associated with subtype of stroke, which is more pronounced in large vessel disease than small vessel disease. These findings correlate with a previous study by Eikelboom et al. They studied 219 ischemic stroke patients with 205 randomly control subjects and found the association was far higher in the patients with large artery disease compared to those with small artery disease[13]. They proposed that the deleterious effect of hyperhomocysteinemia is mediated primarily via a pro-atherogenic effect and less likely due to prothrombotic effect, which might be contributory in the large vessel disease. Contrary to the above observation, Modi M et al, studied hyperhomocysteinemia in 57 ischemic stroke patients in India compared with 30 controls. They could not found any association with the subtype of stroke[14]. They proposed that it could be the smaller number of cases and controls in their study group, exact significance of high homocysteine levels in many subgroups could not be established. There is evidence that hyperhomocysteinemia is both atherogenic and prothrombotic, operating through a variety of potential mechanisms including direct endothelial injury, mitogenic effect on smooth muscle cells, impaired endogenous fibrinolysis, endothelial nitrous oxide response, and alteration in arachinodic acid metabolism[15-17]. The crucial question remains whether plasma homocysteine is directly involved in the pathogenesis of vascular disease or just a marker for increased risk. Vitamin B12 and folate are the co-factors in homocysteine metabolism and have been documented to be strong correlates of plasma homocysteine in many studies and review articles[18,19]. Intervention studies in animals and humans are needed to determine which of the potential mechanisms of homocysteine-associated vascular risk factors are modifiable by targeted vitamin therapy. Recently initiated primary and secondary prevention trials will determine whether lowering of homocysteine levels with vitamin B interventions will lower vascular disease events.

Conclusion

Increased homocysteine level is an important risk factor of the development of ischemic stroke in Thai patients. The effect is more pronounced in large vessel disease than small vessel disease. Further studies of vitamin interventions and association with other risk factors of stroke need to be determined.

References

1. Ministry of Public Health. Burden of disease and injuries in Thailand: priority setting for policy. Nonthaburi: Thai Working Group on Burden of Disease and Injuries, Ministry of Public Health; 2002: A14-6.
2. Stampfer MJ, Malinow MR, Willett WC, Newcomer LM, Upson B, Ullmann D, et al. A prospective study of plasma homocyst(e)ine and risk of myocardial

infarction in US physicians. JAMA 1992; 268: 877-81.
3. Clarke R, Daly L, Robinson K, Naughten E, Cahalane S, Fowler B, et al. Hyperhomocysteinemia: an independent risk factor for vascular disease. N Engl J Med 1991; 324: 1149-55.
4. Kang SS, Wong PW, Cook HY, Norusis M, Messer JV. Protein-bound homocyst(e)ine. A possible risk factor for coronary artery disease. J Clin Invest 1986; 77: 1482-6.
5. Verhoef P, Hennekens CH, Malinow MR, Kok FJ, Willett WC, Stampfer MJ. A prospective study of plasma homocyst(e)ine and risk of ischemic stroke. Stroke 1994; 25: 1924-30.
6. Perry IJ, Refsum H, Morris RW, Ebrahim SB, Ueland PM, Shaper AG. Prospective study of serum total homocysteine concentration and risk of stroke in middle-aged British men. Lancet 1995; 346: 1395-8.
7. Giles WH, Croft JB, Greenlund KJ, Ford ES, Kittner SJ. Total homocyst(e)ine concentration and the likelihood of nonfatal stroke: results from the Third National Health and Nutrition Examination Survey, 1988-1994. Stroke 1998; 29: 2473-7.
8. Taylor LM Jr, DeFrang RD, Harris EJ Jr, Porter JM. The association of elevated plasma homocyst(e)ine with progression of symptomatic peripheral arterial disease. J Vasc Surg 1991; 13: 128-36.
9. 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.
10. Ueland PM, Refsum H, Stabler SP, Malinow MR, Andersson A, Allen RH. Total homocysteine in plasma or serum: methods and clinical applications. Clin Chem 1993; 39: 1764-79.
11. Kang SS, Wong PW, Malinow MR. Hyperhomocyst(e)inemia as a risk factor for occlusive vascular disease. Annu Rev Nutr 1992; 12: 279-98.
12. Sirachainan N, Tapanapruksakul P, Visudtibhan A, Chuansumrit A, Cheeramakara C, Atamasirikul K, at al. Homocysteine, MTHFR C677 T, vitamin B12, and folate levels in Thai children with ischemic stroke: a case-control study. J Pediatr Hematol Oncol 2006; 28: 803-8.
13. 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.
14. Modi M, Prabhakar S, Majumdar S, Khullar M, Lal V, Das CP. Hyperhomocysteinemia as a risk factor for ischemic stroke: an Indian scenario. Neurol India 2005; 53: 297-301.
15. Stein JH, McBride PE. Hyperhomocysteinemia and atherosclerotic vascular disease: pathophysiology, screening, and treatment. off. Arch Intern Med 1998; 158: 1301-6.
16. Diaz-Arrastia R. Homocysteine and neurologic disease. Arch Neurol 2000; 57: 1422-7.
17. Evers S, Koch HG, Grotemeyer KH, Lange B, Deufel T, Ringelstein EB. Features, symptoms, and neurophysiological findings in stroke associated with hyperhomocysteinemia. Arch Neurol 1997; 54: 1276-82.
18. Spence JD. Nutrition and stroke prevention. Stroke 2006; 37: 2430-5.
19. Thompson J. Vitamins and minerals 4: overview of folate and the B vitamins. Community Pract 2006; 79: 197-8.

ระดับโฮโมซีสเตอีนในเลือดกับโรคหลอดเลือดสมองตีบในประเทศไทย

ทัศนีย์ ตันติฤทธิศักดิ์, ธันยชัย สุระ, วรชาติ โมลีฤกษ์ภูมิ, สุชาติ หาญไชยพิบูลย์กุล

ภูมิหลัง: *ภาวะโฮโมซีสเตอีนในเลือดสูงพบว่าอาจเกี่ยวข้องกับการเกิดโรคหลอดเลือดสมองตีบ แต่ยังไม่มีการศึกษานี้ในประเทศไทยอย่างจริงจัง*

วัตถุประสงค์: *การศึกษานี้เพื่อหาความสัมพันธ์ของโรคหลอดเลือดสมองตีบกับระดับโฮโมซีสเตอีนในเลือดและหาค่าผิดปกติในคนไทย รวมทั้งศึกษาความสัมพันธ์ของชนิดของโรคหลอดเลือดสมองกับระดับโฮโมซีสเตอีน*

วัสดุและวิธีการ: *ศึกษาเปรียบเทียบระดับโฮโมซีสเตอีนในเลือดของผู้ป่วยโรคหลอดเลือดสมองตีบ เทียบกับกลุ่มควบคุมที่ไม่มีอาการของโรคหลอดเลือดสมอง ที่มารับการรักษาในสถาบันประสาทวิทยาระหว่าง มิถุนายน พ.ศ. 2543 ถึง พฤษภาคม พ.ศ. 2544 วัดระดับโฮโมซีสเตอีนในเลือดและหาค่าผิดปกติของระดับโฮโมซีสเตอีนในเลือด นำมาวิเคราะห์ทางสถิติ หาความสัมพันธ์ของโรคหลอดเลือดสมอง ชนิดของโรคหลอดเลือดสมองกับระดับโฮโมซีสเตอีนโดยใช้ Logistic regression analyses*

ผลการศึกษา: *ศึกษาเลือดของกลุ่มตัวอย่างทั้งหมด 268 ราย เป็นผู้ป่วยโรคหลอดเลือดสมองตีบ136 ราย และกลุ่มควบคุม 132 ราย ค่าผิดปกติของระดับโฮโมซีสเตอีนในเลือดคือ > 14μmol/L ระดับโฮโมซีสเตอีน มีความสัมพันธ์อย่างมีนัยสำคัญทางสถิติกับโรคหลอดเลือดสมอง คือผู้ที่มีระดับโฮโมซีสเตอีนสูงจะมีโอกาสเป็นโรคหลอดเลือดสมองมากกว่าปกติ 4.277เท่า (odds ratio 4.277; 95%CI 2.551-7.171) และเมื่อตัดปัจจัยเสี่ยงอื่น ๆ ก็พบว่ามีโอกาสมากกว่าปกติ 3.401 เท่า (odds ratio 3.401; 95%CI 1.954-5.922) และพบว่าในผู้ป่วยกลุ่มที่มีหลอดเลือดสมองใหญ่ตีบมีระดับโฮโมซีสเตอีนสูงมากกว่าชนิดหลอดเลือดเล็กตีบอย่างมีนัยสำคัญทางสถิติ*

สรุป: *ภาวะโฮโมซีสเตอีนในเลือดสูงเป็นปัจจัยเสี่ยงของโรคหลอดเลือดสมองตีบและมีผลต่อหลอดเลือดใหญ่ในสมองมากกว่า*