Homocysteine and Risk of Recurrent Stroke

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Background and Purpose—The goals of this work were to investigate whether elevated total homocysteine (tHcy) measured within 24 hours of acute stroke was an independent risk factor for recurrent stroke and to compare levels of tHcy in groups of patients with diagnoses of ischemic and hemorrhagic cerebrovascular events.

Methods—We performed a longitudinal study of 1039 stroke patients (mean age, 75 years). Fasting tHcy was measured the morning after primary admission. Patients were followed up for 15 months.

Results—Serum homocysteine was significantly higher in the 105 patients who experienced a recurrent stroke during the follow-up period than in patients without recurrence. The geometric mean±SD was 13.4±10.7 versus 11.8±7.1 μmol/L (P=0.008), and the mean difference was 1.2 μmol/L [95% confidence interval (CI), 1.05 to 2.3]. In a multiple logistic regression model, tHcy was an independent explanatory variable of recurrent stroke within 15 months (odds ratio, 1.3; 95% CI, 1.1 to 1.5) for each increase in tHcy of 10 μmol/L. At the index event, serum homocysteine was significantly higher in 909 patients with ischemic cerebrovascular events than in 130 patients with intracerebral hemorrhage (geometric mean, 12.1±7.3 versus 10.4±5.2 μmol/L; P<0.001).

Conclusions—The data in this study indicate that elevated tHcy is an independent risk factor for recurrent stroke. (Stroke. 2003;34:1258-1261.)

Key Words: cerebral hemorrhage ■ cerebral infarction ■ cerebrovascular disorders ■ intracerebral hemorrhage ■ risk factors

Identification of modifiable risk factors for stroke may lead to more effective prevention of first and recurrent episodes of cerebrovascular disease. Elevated total serum homocysteine (tHcy) has been associated with an increased risk of vascular disease.1-6 In a meta-analysis of 27 studies, Boushey et al7 reported that for each rise in homocysteine concentration of 5 μmol/L, the odds ratio (OR) for cerebrovascular disease was 1.5 (95% CI, 1.3 to 1.9). In another meta-analysis of 12 studies, Möller et al8 found that plasma or tHcy above the 95th percentile was associated with an OR for cerebrovascular disease of 3.97 (95% CI, 3.07 to 5.12). In a meta-analysis of 27 studies, Boushey et al9 reported that for each rise in homocysteine concentration of 5 μmol/L, the OR for cerebrovascular disease was 1.5 (95% CI, 1.3 to 1.9). In another meta-analysis of 12 studies, Möller et al9 found that plasma or tHcy above the 95th percentile was associated with an OR for cerebrovascular disease of 3.97 (95% CI, 3.07 to 5.12). Prospective studies in which the tHcy value is obtained before the investigation of vascular events are more convincing than studies in which the blood sample is obtained later because tHcy may change as a result of the event.9 Not all prospective cohort studies have confirmed an association between vascular risk and tHcy.10 The Caerphilly study,11 the US Physicians Study,12 and a Finnish study13 did not find an association between tHcy and stroke. In contrast, the British Regional Heart Study,14 the Framingham study15 using nonfasting tHcy, and the Rotterdam study16 found positive associations. We report here a prospective study of risk of recurrent stroke in patients who have had a cerebrovascular event.

Hcy is a sulfur-containing amino acid produced by demethylation of the essential amino acid methionine. tHcy is metabolized via 1 of 2 metabolic pathways: remethylation or transsulfuration. In remethylation, tHcy acquires a methyl group from N-5-methyltetrahydrofolate (MTHF) (this reaction is B12 dependent) or betaine to form methionine again. In transsulfuration, tHcy condenses with serine by cystathionine β-synthase (this reaction is B6 dependent) to form cystathionine, which is then hydrolyzed to form cysteine.17

Hence, inherited deficiencies in enzymes necessary for the metabolism of tHcy can result in elevated blood levels of tHcy, as can deficiencies in required cofactors, folate, B12, B6, and betaine. Other factors known to elevate tHcy are coffee consumption, smoking, use of some drugs, and renal failure.17

The objective of this study was to compare the levels of tHcy measured the morning after admission in patients diagnosed with transient ischemic attack (TIA), ischemic stroke, and intracerebral hemorrhage (ICH) and to evaluate tHcy as a predictor of recurrent stroke within the following 15 months.

Methods

This work is based on 1039 patients who were admitted to the department within 6 hours after onset of cerebrovascular symptoms from February 1, 1998, to October 27, 2000. All patients had a CT scan. Individuals were diagnosed with ischemic stroke if they had a neurological deficit of presumed vascular origin that lasted >24
hours and either a normal CT brain scan or evidence of a recent infarct in the relevant area of the brain. The criteria for TIA was similar, but with symptoms lasting <24 hours. The diagnosis of ICH was based in all cases on CT showing hemorrhage. Cerebrovascular events were also classified according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria.28

Severity of neurological deficit was measured with the Scandinavian Stroke Scale (SSS) on admission. Baseline data on age, sex, diabetes, serum cholesterol, serum triglycerides, atrial fibrillation, and smoking were registered. History of stroke or TIA and history of hypertension were defined as either self-reported or based on information from previous medical records.

Total Hcy was determined as part of the patient’s workup. After an overnight fast, total plasma Hcy was measured on the first morning after admission within 24 hours after stroke onset. The blood sample was kept on ice until centrifugation. For the analysis, Abbott's automated immunofluorescence Hcy test (Abbott ImX method, Abbott Laboratories) was used.

Patients were treated according to the department’s guidelines with antithrombotic and antihypertensive medication, and advice was given concerning lifestyle. Patients with tHcy >15 μmol/L were given advice regarding diet and supplementation with folic acid.

After 15 months, the electronic Hospital Information System was searched for admissions for cerebrovascular disease in each patient. This system covers the eastern part of Denmark, so the coverage was nearly complete. If patients had been admitted elsewhere for their recurrent stroke, they would later be transferred to their local hospital and thereby registered in the system. Nonhospitalized cases of stroke were not included in this analysis. Diagnoses of fatal and nonfatal stroke and TIA were based on discharge letters. The database on which this study was based has been presented to the regional Scientific Ethics Committee, who had no objections to its performance.

Statistical Analyses
Statistical analysis was performed with SPSS for Windows (SPSS Inc). Normal distribution of variables was tested by QQ plot and Kolmogorov-Smirnov's 1-sample test. tHcy results had log-normal distribution and were log10 transformed before parametric analysis. Student's t test was used to compare independent continuous data.

Results
Index Cerebrovascular Event
A total of 1039 patients with an acute cerebrovascular event had tHcy measured. In 702 patients with cerebral infarction and 207 patients with TIA, the geometric mean±SD tHcy was 12.1±7.3 μmol/L. In 130 patients with ICH, the geometric mean tHcy was 10.4±5.2 μmol/L. The difference in geometric mean tHcy between ischemic and hemorrhagic events was statistically significant (P<0.001). The mean difference was 1.2 μmol/L (95% CI, 0.2 to 2.2). The index stroke was classified as large-artery stroke in 13% of patients in whom the geometric mean tHcy was 12.2±7.3 μmol/L, as lacunar stroke in 25% with tHcy of 12.3±4.9 μmol/L, and as cardioembolic stroke in 16% with a mean tHcy of 12.6±7.6 μmol/L.

Not all 1039 index events were first-ever strokes. A prior stroke or TIA was reported by 34% of the 1039 patients and by 36% of the patients with ischemic stroke. Geometric mean

| TABLE 1. Risk Factors of Stroke and Recurrent Stroke in 1039 Patients With Cerebrovascular Disease |
|---------------------------------------------|----------------|---------------|-----|
| Risk Factors                                | Patients With Recurrent Stroke (n=105) | Patients Without Recurrent Stroke (n=934) | P Value* |
| Plasma homocysteine                         | 13.4, SD 10.7  | 11.8, SD 7.1  | 0.008 |
| Diabetes mellitus                           | 13.3%          | 12.4%         | NS   |
| History of stroke or TIA                    | 43.8%          | 31.5%         | 0.015 |
| History of coronary heart disease           | 21.9%          | 17.3%         | NS   |
| Male sex                                    | 58.1%          | 48%           | NS   |
| Active, present smoking                     | 40.0%          | 33.5%         | NS   |
| SSS on admission                            | 44 (35–52)     | 41 (22–52)    | NS   |
| Age                                         | 76 (66–81)     | 75 (64–82)    | NS   |
| History of hypertension                     | 37.6%          | 37.7%         | NS   |
| Total cholesterol, mmol/L                   | 5.4, SD 1.1    | 5.4, SD 1.6   | NS   |

*χ² test, t test, or Mann-Whitney as appropriate. Percent, geometric mean and SD, or median and interquartile range, as appropriate.

NS = not significant.

tHcy was 12.2±7.8 μmol/L in patients who developed ischemic stroke and had a history of stroke or TIA before the index stroke; it was 11.9±6.6 μmol/L in patients without prior cerebrovascular events (P=0.433).

Recurrent Stroke
Recurrent stroke was registered in 105 patients: 97 had suffered an ischemic vascular insult as the index event, and 8 had suffered an ICH. Total Hcy was moderately but significantly higher in patients who developed recurrent stroke than in patients who did not (geometric mean, 13.4±10.7 versus 11.8±7.1 μmol/L; P=0.008; mean difference, 1.2 μmol/L; 95% CI, 1.05 to 2.3) (Table 1). The frequency of prior history of stroke or TIA was also significantly higher in patients who developed a new stroke event during follow-up. The new cerebrovascular events were classified as cerebral infarction in 57.1%, ICH in 9.5%, unspecified stroke in 10.2%, and TIA that required admission in 23.1%. When patients with TIA as the recurrent event were excluded, those who experienced a recurrent stroke had a geometric mean tHcy of 13.6±10.9 μmol/L, and those without recurrence had a mean tHcy of 11.9±7.1 μmol/L (P=0.005; mean difference, 1.2 μmol/L; 95% CI, 1.1 to 1.3) (Table 2).

In a multivariate logistic regression model adjusted for prior stroke or TIA, age, sex, SSS, diabetes mellitus, coronary heart disease, hypertension, and smoking, we found that tHcy was an independent risk factor of recurrent stroke (OR, 1.3; 95% CI, 1.1 to 1.5) for each 10-μmol/L increase in tHcy.

| TABLE 2. Geometric Mean of Total Plasma Homocysteine in Patients With and Without Recurrent Stroke |
|---------------------------------------------|---------------|---------------|-----|
| Ischemic Stroke                             | Hemorrhagic Stroke |
| Recurrent stroke                            | 13.6, SD 10.9 | 10.8, SD 6.5  |     |
| No recurrent stroke                         | 11.9, SD 7.1  | 10.3, SD 5.2  |     |
| t test, P value                            | 0.008         | NS            |     |

NS = not significant.
Prior stroke or TIA was also an independent risk factor (OR, 1.6; 95% CI, 1.1 to 2.5).

Discussion
In our study, elevated $t$Hcy was independently associated with an increased risk of recurrent stroke within the first 15 months after the index cerebrovascular event. Our findings corroborate those of other investigators indicating hyperhomocysteinemia as a risk factor for ischemic stroke. Also, our results are in line with those of Del Ser et al., who found that $t$Hcy exceeding the 75th percentile 3 months after an ischemic stroke was a predictor of vascular events, including stroke recurrence, acute myocardial infarction, deep venous thrombosis, and peripheral arterial disease. The incidence of each vascular illness, however, was too low to be analyzed separately in that study. By focusing solely on cerebrovascular events, our study clearly identifies elevated $t$Hcy as a risk factor for recurrent stroke.

To the best of our knowledge, this is the first study to demonstrate a significant difference in $t$Hcy level between ischemic and hemorrhagic stroke. This difference indicates that the elevated level in ischemic stroke is not just a reaction to the acute illness but reflects the vascular difference between the 2 diseases. Brattström et al and Lindgren et al found no difference in $t$Hcy between patients who had previously had cerebral infarcts and those with ICH. However, their series included only 14 and 12 hemorrhagic stroke patients, respectively.

A strength of our study is that $t$Hcy was measured before the recurrent event. All samples were taken shortly after the index event, and even though they may have been influenced by it, this could hardly affect the risk of a future event.

There are also limitations to our study. We did not have standardized conditions during venipuncture regarding the patient’s posture. This might have elevated $t$Hcy in patients who had been out of bed compared with those who stayed in bed until sampling. However, all samples were obtained early in the morning while most of the patients were still in bed. Furthermore, the ascertainment of patients with recurrent stroke was limited to those who had been hospitalized. This means that we may have missed some cases and thus may have underestimated the effect of hyperhomocysteinemia. Furthermore, there was no systematic follow-up on $t$Hcy concentrations during the observation period. We cannot reliably document the number of patients treated with folic acid after the index stroke because the advice about folic acid was given either to the patient or to the patient’s general physician. Intake of folate might have led to underestimation of the effect of hyperhomocysteinemia.

Some researchers have found an association between hyperhomocysteinemia and subtypes of ischemic stroke. Brattström et al looked at 142 survivors of stroke within 42 weeks after stroke onset; they found no significant differences in $t$Hcy values between the stroke type subgroups. Lindgren et al found that $t$Hcy was higher in the total anterior circulation infarct group than in other stroke types, which was partly explained by higher age in that group. Eikelboom et al studied 219 cases and 205 controls and found significantly higher plasma $t$Hcy in cases with stroke caused by large-artery and small-artery disease compared with control subjects. Tan et al found higher $t$Hcy levels in large-artery stroke compared with small-artery strokes in patients <50 years of age. In our study, we did not find any difference among the different ischemic stroke types, but ischemic stroke as a group had higher $t$Hcy than hemorrhagic stroke.

It can be debated whether $t$Hcy is a risk factor or only a risk marker for vascular disease, but the observation that hyperhomocysteinemia resulting from defects in 1 of 3 different enzymes (cystathionine synthase, MTHF homocysteine methylytransferase, or methylenetetrahydrofolate reductase) in each case causes serious vascular disease supports the idea of a pathogenetic role for $t$Hcy.

In addition, a possible effect of $t$Hcy on carotid artery stenosis has been examined by ultrasonography. Cross-sectional studies have shown that elevated levels of $t$Hcy are associated with extracranial carotid artery stenosis. and treatment with $t$Hcy-lowering agents such as folic acid, pyridoxine, and cyanocobalamin has been shown to slow the progression of carotid plaque in patients. A recent meta-analysis by the Homocysteine Studies Collaboration confirmed homocysteine as a risk factor for first events of stroke and coronary heart disease. A reduction in homocysteine level by $\approx 3 \mu$mol/L was associated with 19% lower stroke risk (OR, 0.81; 95% CI, 0.69 to 0.95). A reduction in homocysteine level of $\approx 3 \mu$mol/L is what can be achieved on average by folic acid supplementation. In our study, the OR of recurrent stroke was 1.3 per 10-$\mu$mol/L increase in $t$Hcy, whereas in studies considering first stroke, an increase in $t$Hcy of 5 $\mu$mol/L is associated with a similar OR. The magnitude of the effect thus seems greater in primary events than in recurrent events.

All the studies mentioned above support the hypothesis that $t$Hcy plays a role in the pathogenesis of vascular disease.

In conclusion, the present data suggest that elevated $t$Hcy is an independent risk factor for recurrent stroke. Measurement of $t$Hcy may become an integrated part of the workup of stroke patients. Elevated $t$Hcy values may easily be reduced by vitamin supplementation, which may be an important asset in future secondary stroke prevention. Ongoing intervention studies will clarify whether treating hyperhomocysteinemia can reduce the risk of stroke and recurrent stroke.

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