Relation of Plasma Homocyst(e)ine to Cerebral Infarction and Cerebral Atherosclerosis

Jun-Hyun Yoo, MD, PhD; Chin-Sang Chung, MD, PhD; Soo-Sang Kang, MD, PhD

Background and Purpose—A number of investigations support the theory that the elevated plasma homocyst(e)ine is associated with occlusive vascular disease. The aim of this study is to examine whether moderate hyperhomocyst(e)inemia is an independent risk factor for cerebral infarction. In addition, we examined the association between plasma homocyst(e)ine and the severity of cerebral atherosclerosis.

Methods—We conducted a hospital-based case-control study with 140 male controls and 78 male patients with nonfatal cerebral infarction, aged between 39 and 82 years. Plasma homocyst(e)ine levels were analyzed in 218 subjects. Fifty-five patients were evaluated for cerebral vascular stenosis by MR angiography.

Results—The mean plasma level of homocyst(e)ine was higher in cases than in controls (11.8±5.6 versus 9.6±4.1 μmol/L; P=0.002). The proportion of subjects with moderate hyperhomocyst(e)inemia was significantly higher in cases than in controls (16.7% versus 5.0%; P=0.004). Based on the logistic regression model, the odds ratio of the highest 5% of homocyst(e)ine levels in control group was 4.17 (95% confidence interval, 3.71 to 4.71) (P=0.0001). After additional adjustment for total cholesterol, hypertension, smoking, diabetes, and age, the odds ratio was 1.70 (95% confidence interval, 1.48 to 1.95) (P=0.0001). The plasma homocyst(e)ine levels of patients having vessels with 3 or 2 stenosed sites were significantly higher than those of patients having vessels with 1 stenosed site or normal vessels (14.6±1.4, 11.0±1.4 versus 7.8±1.5, 8.9±1.4 μmol/L respectively; P<0.02). Multiple logistic regression analysis revealed that moderate hyperhomocyst(e)inemia was significantly associated with the number of stenosed vessels (P=0.001).

Conclusions—These findings suggest that moderate hyperhomocyst(e)inemia is an independent risk factor for cerebral infarction and may predict the severity of cerebral atherosclerosis in patients with cerebral infarction. (Stroke. 1998;29:2478-2483.)

Key Words: atherosclerosis ■ cerebral arteries ■ cerebral infarction ■ homocyst(e)ine

Stroke remains the leading cause of death in Korea.1 The National Statistical Annual Report shows relatively low mortality (13/100 000 population) due to coronary artery disease, while showing markedly higher mortality (80/100 000 population) due to cerebrovascular disease, in which cerebral infarction is predominantly involved.1 This is in contrast to parts of the Western world, in which there is higher mortality due to coronary artery disease. The conventional risk factors for cerebral infarction in Koreans include hypertension, smoking, hypercholesterolemia, diabetes mellitus, and aging.2 However, these do not account for all the cases of ischemic stroke, because many patients have events without having any of these conventional risk factors.

Homocysteine is a sulphydryl amino acid that is readily oxidized to homocystine and homocysteine-cysteine mixed disulfide in the plasma. Homocyst(e)ine refers to the sum of homocysteine, homocystine, and the homocysteine-cysteine mixed disulfide, in both the free and protein-bound forms.3 Based on the findings observed in patients with homocystinuria, McCully4 postulated that hyperhomocyst(e)inemia may play a role in the pathogenesis of atherothrombotic vascular disease. In patients with inherited severe hyperhomocyst(e)inemia,5 thromboembolic events were observed in one half of the patients by age of 29 years; 32% of those showed cerebrovascular events, an increased tendency toward cerebral infarction, premature arteriosclerosis, and occlusive arterial disease.5 Many epidemiological studies have suggested that even moderate hyperhomocyst(e)inemia is also associated with the occurrence of occlusive vascular disease,5,6 including ischemic heart disease,7-9 stroke,10-16 and peripheral arterial occlusive disease.17,18 Moreover, a significant correlation of homocyst(e)inemia to extracranial carotid ath-
erosclerosis\textsuperscript{19,20} has been seen, and Ubbink et al\textsuperscript{21} reported a possible correlation to the severity of coronary atherosclerosis. The actions of homocyst(e)ine on atherothrombogenesis, such as vascular endothelial dysfunction or injury,\textsuperscript{22,23} proliferation of vascular smooth muscle cell,\textsuperscript{24} and altered blood coagulation,\textsuperscript{25,26} support these studies. These findings suggest that hyperhomocyst(e)inemia may contribute to the progression of cerebral atherosclerosis. However, the relationship between plasma homocyst(e)ine and the severity of cerebral atherosclerosis has not yet been reported.

The aim of our study was to examine whether moderate hyperhomocyst(e)inemia is an independent risk factor for cerebral infarction and whether it is related to the severity of cerebral vascular stenosis in patients with cerebral infarction.

Subjects and Methods

Subjects
Study subjects were all unrelated Korean males. Stroke patients between the ages of 39 and 82 years who attended the stroke clinic in the Department of Neurology at Samsung Medical Center (SMC), Seoul, Korea, were recruited to participate in the study from June through September 1996. One hundred thirty-eight patients consented to blood sampling for homocyst(e)ine determination. They had survived a stroke during the previous year and maintained a regular diet and daily activities with or without partial aids. They all resided in their house with their wife or members of their family; there were no institutionalized patients. Four patients had a history of multiple stroke. Evaluation of brain by MRI or CT was carried out previously for the patients who consented to undergo study. Chest x-ray, ECG, serum electrolytes, fasting serum glucose, lipids, creatinine, uric acid, liver function test, thyroid hormone, thyroid-stimulating hormone, and complete blood counts were examined. The social and medical histories of the control subjects were obtained by interviews with the investigator. Forty-five individuals were excluded from the study according to criteria identical to that for the cases. In addition, individuals recruited for control were screened for evidence of symptomatic atherosclerotic disease by the treadmill test or carotid duplex scan if they had chest pain or carotid bruits accompanied by a history of hypertension, diabetes mellitus, or smoking. Seventeen individuals with positive results in the treadmill test and 10 individuals with intimal thickening by duplex scan were also excluded. A total of 140 subjects were selected as the control group. The study was approved by the Institutional Review Board of SMC, and informed consent was obtained from all the participants.

Hypercholesterolemia was defined as a fasting serum total cholesterol level $>6.2$ mmol/L. Hypertension was considered to be present in subjects who were currently taking antihypertensive medication or in 2 control subjects with blood pressure taken by random zero sphygmomanometer of $\geq 140$ mm Hg systolic and/or $\geq 90$ mm Hg diastolic at least twice consecutively. Diabetes was considered to be present in subjects who were currently using oral hypoglycemic agents or insulin, or in 1 case and 2 control subjects with fasting plasma glucose $>7.8$ mmol/L and postprandial 2-hour glucose $>11.1$ mmol/L.

Laboratory Determination of Plasma Samples

Blood was drawn from the antecubital vein of each subject during a state of fasting and collected into Vacutainer K3 EDTA tubes (Beckton Dickinson). Blood for complete blood count and blood chemistry profiles was examined by automatic analyzer (HS-330, HITACHI 747). The evacuated tube was immersed in ice water up to the neck and transported to the laboratory at the temperature of $0^\circ$C. Samples were centrifuged at $4^\circ$C within 3 hours and stored with aliquots of plasma at $-75^\circ$C until the time of analysis. The samples from the cases and controls were handled together in a double-blind manner during the time of analysis.

Plasma homocyst(e)ine levels were determined by the method of Vester and Rasmussen.\textsuperscript{27} The reductant, derivatizing agent, internal standard, and DL-homocysteine were obtained from Sigma Chemical Co. The HPLC system consisted of the following: Waters 510 HPLC pumps, Waters 717 plus Autosampler, Waters 474 scanning fluorescence detector, Merck LiChrosper 100 RP-18 column (4×125 mm, 5-μm particles), and Merck LiChrosor 100 RP-18 guard column. The coefficient of variation was less than 3%. Plasma folate and vitamin B$\textsubscript{12}$ were determined by radioimmunoassays (DPC).

Statistical Analysis

SAS (version 6.12) statistical software was used for the analysis. Values of continuous variables were expressed as mean±SD. Comparisons between mean values and the proportions of various vascular risk factors between cases and controls were carried out using t tests and $\chi^2$ tests. Because plasma homocyst(e)ine and serum triglyceride levels were not normally distributed, natural logarithmic transformation and geometric means were used. Normality of the distribution was tested using Shapiro-Wilk statistics. The Shapiro-Wilk W test changed from $W=0.83$ to $W=0.97$ for raw and log transformed plasma homocyst(e)ine levels, respectively.

The homocyst(e)ine values among the number of stenosed arteries were compared using ANOVA, followed by the application of the Duncan method for multiple comparisons. The pairs between individual means were compared using t tests.

Association between plasma homocyst(e)ine and stroke risk factors were tested by calculating Pearson correlation coefficients. The
cutoff point for moderate hyperhomocyst(e)inemia was 15.5 μmol/L, which represents the top 95th percentile of the distribution of the control subjects.

A multiple logistic regression model was used to estimate the odds ratio (OR) for cerebral infarction and the 95% confidence interval (CI). To adjust for the effects of other risk factors, the serum total cholesterol (cutoff level, 6.2 mmol/L), homocyst(e)ine (cutoff level, 15.5 μmol/L), presence or absence of hypertension, smoking status, diabetes mellitus, and age (≥65 years) were fitted as independent dichotomous variables, and the presence or absence of cerebral infarction was regarded as the dependent variable.

The possible interactions between plasma homocyst(e)ine levels and hypertension or other risk factors were explored in stratified analyses and by comparison of the goodness-of-fit of logistic regression models, with and without interaction terms, by the likelihood ratio test. All values for the probability were analyzed by regression models, with and without interaction terms, by the analyses and by comparison of the goodness-of-fit of logistic regression models.

### Results

#### Distribution of Plasma Homocyst(e)ine Levels and Laboratory Data

The demographic and clinical data of 78 cases and 140 controls are shown in Table 1, with a comparison of conventional risk factors. The mean age was 56.2±10.1 years (range, 39 to 82 years) and 61.1±8.9 years (range, 39 to 82 years) in cases and controls, respectively.

Plasma homocyst(e)ine concentration was determined in 218 subjects. The mean concentrations in cases were 11.8 μmol/L; maximum was 42.1; minimum was 4.7; 95th percentile was 22.2; and the mode was 9.2 μmol/L in raw value. Mean plasma homocyst(e)ine was higher in cases than in controls (11.8±5.6 versus 9.6±4.1 μmol/L; P = 0.002).

The prevalence of moderate hyperhomocyst(e)inemia (≥15.5 μmol/L) was substantially higher among patients with cerebral infarction than among normal controls (16.7% versus 5.0%; P = 0.004). Frequency of hypertensives, diabetics, and smokers was higher among case subjects than control subjects (P < 0.05). Mean age, levels of total cholesterol, HDL, LDL, creatinine, uric acid, and smoking amount were not significantly different between patients with and without hyperhomocyst(e)inemia.

#### Correlation Between Plasma Homocyst(e)ine and Lipids, Folate, and Vitamin B12

There was no significant correlation between plasma homocyst(e)ine levels and age, body mass index, levels of serum cholesterol, LDL cholesterol, HDL cholesterol, triglyceride, creatinine, and uric acid. However, plasma homocyst(e)ine levels exhibited a strong inverse association with plasma folate levels in controls (r = −0.37; P = 0.002) and cases (r = −0.13; P = 0.02). Vitamin B12 did not show correlation to plasma homocyst(e)ine in cases or controls (P < 0.05) (Table 2).

#### Association Between Plasma Homocyst(e)ine and Cerebral Infarction

Logistic regression analysis was used to calculate the OR with 95% CI adjusted for the effects of the conventional stroke risk factors. In univariate analysis, the OR of hyperhomocyst(e)inemia for cerebral infarction was 4.17 (95% CI, 3.71 to 4.71) (P = 0.0001), but after adjustment for risk factors associated with increased risk of stroke, such as total cholesterol, hypertension, smoking status, diabetes, and age, the OR was 1.70 (95% CI, 1.48 to 1.95) (P = 0.0001) (Table 3).

Stratified analyses were conducted to find out possible interaction between hyperhomocyst(e)inemia and risk factors, such as hypertension, smoking status, and age. Hyperhomocyst(e)inemia was associated with increased risk of cerebral infarction in hypertensives, with an OR of 1.79 (95% CI, 1.61 to 1.97), than in normotensives, with an OR of 1.32 (95% CI, 1.18 to 1.48). However, there was no significant difference on testing for interaction (P < 0.05). The association between hyperhomocyst(e)inemia and the risks for stroke of old age and smoking could not be tested due to limited number of subjects.
TABLE 3. Adjusted Odds Ratios of Cerebral Infarction Based on Multiple Logistic Regression Analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypercholesterolemia</td>
<td>0.12</td>
<td>1.13</td>
<td>0.98–1.32</td>
<td>0.11</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.40</td>
<td>4.06</td>
<td>3.74–4.39</td>
<td>0.0001</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.37</td>
<td>3.94</td>
<td>3.49–4.44</td>
<td>0.0001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.06</td>
<td>2.89</td>
<td>2.61–3.19</td>
<td>0.0001</td>
</tr>
<tr>
<td>Old age</td>
<td>0.65</td>
<td>1.92</td>
<td>1.75–2.14</td>
<td>0.0001</td>
</tr>
<tr>
<td>Hyperhomocyst(e)inemia</td>
<td>0.53</td>
<td>1.70</td>
<td>1.48–1.95</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Hypercholesterolemia indicates serum cholesterol level ≥6.2 mmol/L; hyperhomocyst(e)inemia, plasma homocyst(e)ine level ≥15.5 μmol/L; and elderly, ≥65 years of age.

Association Between Plasma Total Homocyst(e)ine Concentration and the Severity of Cerebral Vascular Stenosis

The proportions of patients with 1-, 2-, and 3-site stenosis were 34.5%, 20%, 9.1%, respectively. The mean ages of these patient groups had similar values (62.7±9.9, 62.3±7.1, and 65.2±4.4 years, respectively; P<0.05). Extracranial carotid stenosis was observed in 12% of overall stenosis. In intracranial arterial stenosis, 64% of overall stenosis was found in circle of Willis (internal carotid artery, 17.3%; middle cerebral artery, 32%; anterior cerebral artery, 5.6%; posterior cerebral artery, 9.3%), and 24% was found in the vertebral and basilar arteries. The mean homocyst(e)ine concentrations of patients with 3 or 2 stenotic vessels were higher than those of patients with a single stenotic vessel (14.6±1.4, 11.0±1.4 versus 7.8±1.5 μmol/L, respectively; P=0.002) (see the Figure). There was no difference between the mean homocyst(e)ine level in patients with single stenotic vessel and subjects without stenosis (7.8±1.5 versus 8.9±1.4 μmol/L; P>0.05). Patients who underwent MRA were dichotomously divided into a mild group (normal vessels or 1 stenotic vessel) and a severe group (2 or 3 stenotic vessels), according to the severity of vascular stenosis. We then conducted a linear logistic regression analysis to assess the association between homocyst(e)ine level and the severity of cerebral vascular stenosis (no and 1 stenotic vessel versus 2 and 3 stenotic vessels). After controlling for other conventional risk factors such as hypertension, hypercholesterolemia, smoking, diabetes and old age, elevated homocyst(e)ine levels remained significantly related to the severity of stenosis (regression coefficient β=0.16; SE=0.04; P=0.0001).

Discussion

Elevated plasma homocyst(e)ine has been increasingly recognized as a risk factor for occlusive vascular disease.3,7–18 In the present study, we investigated plasma levels of homocyst(e)ine in patients with cerebral infarction in an ethnic group with a high incidence of stroke, and as a result, plasma homocyst(e)ine levels of patients were significantly higher compared with healthy controls. This finding is consistent with the previous studies.10,11,13,14,16,29 The prevalence of moderate hyperhomocyst(e)inemia was 3.3-fold higher among patients with cerebral infarction compared with normal controls. Such a result is within the range of results in previous studies, from 18.5% (1.8-fold)15 to 22% (4.4-fold)16 and 42% (6.6-fold).11 In our study, plasma homocyst(e)ine levels appeared to be lower than those of Caucasians. Plasma homocyst(e)ine level could be determined by genetic or environmental factors and in conjunction with both. The prevalence of the thermolabile homozygous variant of methylenetetrahydrofolate reductase is 11.8% in healthy Koreans,29 which is similar to that in Caucasians. However, the Korean diet consists of 12.5% protein, 22.8% fat, 64.7% carbohydrate in terms of energy ratio from a macronutrient supply.30 The national average for animal food intake is 229 g/d per person (21.6%); on the other hand, the vegetable intake, including boiled rice as the main food, is 833 g/d per person (78.4%).30 Such a relatively low level of meat consumption is probably related to lower plasma homocyst(e)ine levels.

The first aim of this study was to examine whether moderate hyperhomocyst(e)inemia is an independent risk factor for cerebral infarction. Plasma homocyst(e)ine levels were not correlated to continuous variables, such as age, lipid profiles, and uric acid, or to dichotomous variables, such as hypertension, smoking, and diabetes mellitus (P<0.05). Also, no difference in the levels of serum cholesterol, HDL cholesterol, LDL cholesterol, uric acid, and smoking amount was observed between those with and without hyperhomocyst(e)inemia (data not shown). After additional adjustment for total cholesterol, hypertension, smoking, diabetes, and age, multiple logistic regression analysis revealed that men with moderate hyperhomocyst(e)inemia were at a 1.7-fold higher risk of cerebral infarction.

It has been suggested that the effect of homocyst(e)ine was more pronounced in the presence of other risk factors. Malinow et al19 showed that the association between plasma homocyst(e)ine and thickening of the carotid intimal-medial wall was stronger in hypertensive than in normotensive subjects. We observed an increased risk of stroke in hypertensives over normotensives, though there was no significant difference, as presented in the study of Perry et al.13 Araki et al28 suggested that hyperhomocyst(e)inemia increased the risk for arteriosclerotic cerebral infarction but did not present the OR or the difference between cases and controls according to hypertension. To the contrary, the data from the Physician’s Health Study12 showed an increased OR of
hyperhomocyst(e)inemia for stroke in normotensive subjects and in men ≤60 years. The interaction between homocyst(e)ine and stroke risk factors, such as hypertension, smoking, diabetes mellitus, and aging, needs to be investigated by further studies with larger sample sizes.

While hypercholesterolemia is a strong risk factor for coronary artery disease, the association with ischemic stroke remains uncertain. In our data, the proportion of hypercholesterolemia was higher in case than in control subjects, but it did not significantly differ in the cutoff level of 6.2 mmol/L (9% versus 3.6%; \( P = 0.09 \)). Using a cutoff level of 5.7 mmol/L (25.6% versus 18.6%; \( P = 0.22 \)) or continuous variable, there was also no significant correlation between hypercholesterolemia and cerebral infarction (Table 3).

In our study, there were some limits in the study design. Fatal cases were not included. Participants were from a volunteer group in the ambulatory outpatient care unit. Moreover, homocyst(e)ine levels in stroke patients in rehabilitation at the time of blood sampling might be underestimated compared with those from before stroke, owing to the change in dietary habit, such as increased vegetable intake, and reduced meat or alcohol consumption. It was known that chronic alcohol drinking was positively related to tissue folate content or plasma homocyst(e)ine level. In the selection of control group, it might be ideal to compare 2 control groups: one without cerebral infarction and the other without evidence of atherosclerosis. We excluded subjects with a positive treadmill test or thickened carotid intima from this study. When we determined plasma homocyst(e)ine levels in such subjects, the mean value was 8.9 ± 3.8 μmol/L, similar to that of control group.

In our previous data, we observed that individuals with moderate hyperhomocyst(e)inemia were at a 1.4-fold higher risk of coronary artery disease and also related to the severity of coronary artery disease. These findings suggest that moderate hyperhomocyst(e)inemia is associated with symptomatic atherosclerotic disease. Atherosclerotic lesion of the coronary or cerebral arteries is one of the underlying conditions of the pathogenesis of myocardial or cerebral infarction. Although there is a large body of evidence relating high levels of serum cholesterol with coronary atherosclerosis, the relation between serum cholesterol and cerebral atherosclerosis is less clear.

The second aim of this study was to explore the association between plasma homocyst(e)ine and the severity of cerebral atherosclerosis. To assess the relation of homocyst(e)ine concentration among the patients with vascular stenosis, MRA was used as a neuroimaging technique. MRA is noninvasive and can detect vascular occlusive change with high sensitivity and specificity. A dose-response relationship between plasma homocyst(e)ine level and the number of significantly stenosed vessels was observed. The relative frequency of severe degree of cerebral atherosclerosis varies in relation to risk factors. Using a logistic regression model in our study, after adjusting for the conventional risk factors for stroke, such as hypertension, smoking, diabetes mellitus, hypercholesterolemia, and old age, moderate hyperhomocyst(e)inemia was significantly related to the severity of atherosclerosis. Our findings in patients with cerebral infarction were consistent with the fact that intracranial atherosclerosis is more prominent than extracranial atherosclerosis in Oriental patients. In the Atherosclerosis Risk in Communities study, it was shown that fasting plasma levels of homocyst(e)ine were significantly higher in asymptomatic case subjects with thickened intimal-medial carotid walls than in control subjects. The OR for having a thickened carotid wall was 3.15 (\( P = 0.001 \)) for subjects in the top quintile of plasma homocyst(e)ine level compared with those in the bottom quintile. These findings suggest that moderate hyperhomocyst(e)inemia may contribute to the development and progression of cerebral atherosclerosis. The mechanisms of the atherothrombogenic action of homocysteine and its derivatives support these observations. The oxidation of homocyst(e)ine promotes the oxidation of low-density lipoprotein cholesterol, which causes injury to vascular endothelial cells and leads to endothelial dysfunction. Homocysteine also promotes vascular smooth muscle cell growth, and increases in platelet thromboxane A2 production, platelet aggregation, and factor V activity were observed. The cerebral infarction is the consequence of cellular necrosis due to the perfusion defect of brain. Neurotoxicity of homocyst(e)ine may play a role in the pathogenesis of cerebral infarction. Recently, neurotoxicity of homocysteine through overstimulation of N-methyl-D-aspartate receptors was observed.

Hyperhomocyst(e)inemia has been known to be associated with premature atherosclerosis. However, many studies have shown the association between hyperhomocyst(e)inemia and cerebrovascular disease in subjects, including the elderly. Several studies, whose subjects are similar in age to those in our study, have shown significantly higher homocyst(e)ine levels in cases with cerebrovascular disease than in controls. These results in the elderly suggest that the lifelong exposure to moderate hyperhomocyst(e)inemia may predispose the elderly to ischemic stroke.

The present findings are consistent with the hypothesis that moderate hyperhomocyst(e)inemia is independent risk factor of nonfatal cerebral infarction and related to the severity of cerebral atherosclerosis. It is not yet known if the reduction of plasma homocysteine level will be effective for primary prevention of ischemic stroke. Elevated homocyst(e)ine levels encountered in patients with vascular disease can be reduced to normal by folate, betaine, or vitamin B6 supplementation. Further studies are needed to confirm the effect on the prevention of cerebral infarction or cerebrovascular atherosclerosis by lowering plasma homocyst(e)ine.

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