Association of Plasma Homocysteine Concentration With Atherosclerotic Carotid Plaques and Lacunar Infarction

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Background and Purpose—Higher plasma total homocysteine (tHcy) levels have been associated with carotid atherosclerosis and cerebral infarction in whites. However, data regarding such associations are limited for Asians. This study examined associations between tHcy levels and severity of carotid atherosclerosis in Japanese subjects. Additionally, because lacunar infarction is the most prevalent type of ischemic stroke in Japan, we also investigated its associations with tHcy levels.

Methods—The subjects were 152 Japanese patients (age, 66.2±11.0 years) at our hospital. Using ultrasound, we evaluated severity of carotid atherosclerosis by plaque score, which is defined by the sum of all plaque (intima-media thickness ≥1.1 mm) height in bilateral carotid arteries. In 112 of 152 patients, the existence of lacunar infarction was evaluated on brain MRI scans.

Results—A moderate linear association was found between tHcy levels and plaque score (r=0.48, P<0.0001). Moreover, tHcy level was associated with plaque score (β=0.26, P<0.001) independently of traditional atherosclerotic risk factors. In logistic regression analyses, each 1-μmol/L-higher tHcy level was associated with a 1.37-fold-higher [95% confidence interval (CI), 1.19 to 1.58] likelihood for lacunar infarction, increasing the likelihood by 1.22-fold (95% CI, 1.04 to 1.43) independently of traditional atherosclerotic risk factors.

Conclusions—Higher tHcy levels appear to have associations with increased severity of carotid atherosclerotic plaques and prevalent lacunar infarction in the Japanese. Larger prospective studies are necessary to establish whether higher tHcy levels serve as a harbinger for insidious carotid and cerebrovascular diseases. (Stroke. 2002;33:1493-1496.)

Key Words: carotid arteries | homocyst(e)ine | lacunar infarction | risk factors

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Elevated plasma total homocysteine (tHcy) levels have been indicated as a risk factor for coronary heart disease,1–3 ischemic stroke,4–6 and peripheral artery disease.7,8 Also, studies have related tHcy levels to carotid atherosclerosis as a surrogate endpoint for cardiovascular diseases.9–11 However, most of these findings were derived from white populations, and whether such findings also apply for Asians remains to be determined. Additionally, little data are available regarding the relationship between tHcy levels and lacunar infarction, the most prevalent type of ischemic stroke in the Japanese.12

To quantify carotid atherosclerosis, we have used plaque score and demonstrated its potential utilities for stroke risk assessment.13,14 This study examined the associations between carotid plaque score and tHcy levels in the Japanese and investigated whether higher tHcy levels are associated with prevalent lacunar infarction as evidenced by brain MRI.

Subjects and Methods

Subjects
Subjects for this investigation were enrolled from 184 patients consecutively admitted to the National Osaka (Japan) Minami Hospital. All patients had at least 1 of the following diseases: hypertension, diabetes mellitus, hyperlipidemia, ischemic stroke, or arteriosclerosis obliterans. Stroke was diagnosed in 77 of the 184 patients, including 34 lacunar infarction, 21 atherothrombotic infarction, 11 cardioembolic infarction, and 11 unclassified stroke.

The exclusion criteria for the current study were malignant diseases, hypothyroidism, chronic renal failure (serum creatinine concentration >0.26 mmol/L), recent myocardial infarction, major surgery, and the use of anticonvulsants, multivitamins, methotrexate, or nitrous oxide. Additionally, because the implication of atherosclerosis is not clear for cardioembolic infarction and unclassified stroke, patients with these conditions were also excluded.

Among the 184 patients, 32 patients met either of the above criteria and were excluded. Consequently, a total of 152 Japanese patients (age, 66.2±11.0 years), 81 women and 71 men, were
involved in this study. Baseline characteristics of the 152 patients are shown in Table 1.

Informed consent was obtained from all subjects before carotid and brain MRI examinations were performed.

### Carotid Ultrasonography

Carotid ultrasonography was performed with the use of a linear-array 7.5-MHz transducer (Hewlett Packard Sonos 5500). In accordance with our prior study, severity of carotid atherosclerosis was evaluated by plaque score. Briefly, the patients lay in the supine position in a dark room, and the examinations were done with the head held in the midline position or slightly tilted to either side. Initially, the common and internal carotid arteries were scanned cross sectionally and longitudinally, through which the distribution of atheromatous plaques [focal increases in intima-media thickness (IMT)] was roughly evaluated. During the initial scanning, optimal insonation angles were determined for the estimation of respective plaque heights, and measurements were performed on the frozen frame perpendicular to the vascular walls. Bilateral carotid arteries were examined with the same procedures. Subsequently, plaque score was calculated by summing up the height of all plaques located in bilateral carotid arteries. The length of individual plaques was not considered in the calculation of this score.

### Evaluation of Plasma tHcy levels

Blood was drawn from fasting subjects on the day of carotid examination. Venous blood samples were collected in tubes containing EDTA, centrifuged within 60 minutes, and stored at −20°C to avoid a false elevation in tHcy concentration as the result of its release from red blood cells. The tHcy level determined by high-performance liquid chromatography was considered to be the sum of the homocysteine, the homocysteinyl moieties of homocysteine, and the cysteine-homocysteine mixed disulfide levels, regardless of whether they were free or protein bound. For stroke patients, tHcy levels were measured >8 weeks after the events because the levels can temporally elevate after stroke.

### Evaluation of Lacunar Infarction

Brain MRI scans were performed for 112 of the 152 subjects. Multislice spin-echo images (10-mm slice thickness, 1.0-T scanner, Siemens Co) were obtained with a repetition time (TR) of 5000 ms and an echo time (TE) of 100 ms for T2-weighted images and a TR of 370 ms and a TE of 17 ms for T1-weighted images. Lacunar infarction was defined as a hyperintense area on T2-weighted images (5 mm ≤ diameter <15 mm) that was visible as a low-signal intensity on T1-weighted images. To exclude enlarged perivascular spaces, lesions <5 mm were not counted as infarctions, as described by Braffman et al.

### Data Analyses

Associations of carotid plaque score with tHcy and atherosclerotic risk factors were examined by univariate linear regression analyses and unpaired t test, followed by multiple linear regression analyses. The risk factors considered in this study were hypertension (casual blood pressure ≥160/95 mm Hg or on medication), diabetes mellitus (fasting plasma glucose ≥7.77 mmol/L, glycosylated hemoglobin ≥6.4%, or on medication), hyperlipidemia (serum total cholesterol ≥5.70 mmol/L or on medication), and smoking. Patients were categorized as smokers when they smoked at least 1 cigarette per day and as nonsmokers when they had never smoked or had stopped smoking >3 years ago. The ability of plasma tHcy levels to stratify the likelihood for lacunar infarction was examined by logistic regression analyses. A value of P<0.05 was considered statistically significant. Results are reported as mean±SD unless otherwise specified. SPSS 9.0 statistical software was used for all analyses.

### Results

Plaque score had linear associations with tHcy (r=0.482, P<0.0001; the Figure) and age (r=0.507, P<0.0001) but not with BMI. Although quadratic, cubic, and exponential terms were also examined, the explicable variance was similar to that obtained by linear regression. Also, plaque score was higher in men than in women (8.1±5.5 versus 4.4±4.4, P<0.05) and in smokers than in nonsmokers (7.5±5.9 versus 5.6±4.9, P<0.05), whereas significant differences were not found in the diagnoses of hypertension, hyperlipidemia, and diabetes.

To further examine associations of plaque score with tHcy and atherosclerotic risk factors, multiple linear regression analysis was performed. A higher tHcy level was found to be associated with plaque score independently of age, sex, and other traditional atherosclerotic risk factors (Table 2).

### Table 1. Demographic and Clinical Characteristics of 152 Subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>66.2±11.0</td>
</tr>
<tr>
<td>Male sex, %</td>
<td>46.7</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>23.1±3.2</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>69.5</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>20.5</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>32.0</td>
</tr>
<tr>
<td>Hyperlipidemia, %</td>
<td>32.6</td>
</tr>
<tr>
<td>Homocysteine, μmol/L</td>
<td>9.43±3.60</td>
</tr>
<tr>
<td>Fibrinogen, g/L</td>
<td>2.93±0.791</td>
</tr>
<tr>
<td>Creatinine, mmol/L</td>
<td>0.083±0.033</td>
</tr>
<tr>
<td>Cerebrovascular diseases, %</td>
<td>36.2</td>
</tr>
<tr>
<td>Plaque score</td>
<td>6.2±5.3</td>
</tr>
</tbody>
</table>

### Table 2. Determinants of Plaque Score

<table>
<thead>
<tr>
<th>Determinant</th>
<th>B</th>
<th>β</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocysteine (μmol/L)</td>
<td>0.370</td>
<td>0.260</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (y)</td>
<td>0.177</td>
<td>0.372</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex (male=1)</td>
<td>2.369</td>
<td>0.226</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.037</td>
<td>0.090</td>
<td>0.18</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.712</td>
<td>0.063</td>
<td>0.51</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.576</td>
<td>0.044</td>
<td>0.37</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>−0.127</td>
<td>−0.011</td>
<td>0.86</td>
</tr>
</tbody>
</table>

No significant interactions were observed between significant regressors.
TABLE 3. Logistic Regression Analysis for Predicting Lacunar Infarction

<table>
<thead>
<tr>
<th></th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocysteine (µmol/L)</td>
<td>1.22</td>
<td>1.04–1.43</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Age (/10 yr)</td>
<td>1.61</td>
<td>1.02–2.53</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Sex (male=1)</td>
<td>4.58</td>
<td>1.84–11.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>2.01</td>
<td>0.74–5.44</td>
<td>0.17</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.85</td>
<td>0.29–2.49</td>
<td>0.77</td>
</tr>
<tr>
<td>Smoking</td>
<td>2.15</td>
<td>0.85–5.48</td>
<td>0.11</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>1.01</td>
<td>0.41–2.53</td>
<td>0.98</td>
</tr>
</tbody>
</table>

OR indicates odds ratio; CI, confidence interval.

Subsequently, the ability of tHcy levels to stratify the likelihood for lacunar infarction was examined by logistic regression analyses. Each 1-µmol/L-higher tHcy level was associated with a 1.37-fold-higher (95% confidence interval (CI), 1.19 to 1.58] likelihood for lacunar infarction, increasing the likelihood by 1.22-fold (95% CI, 1.04 to 1.43) independently of traditional atherosclerotic risk factors (Table 3).

**Discussion**

Elevated tHcy levels have attracted much attention in recent years as a potential risk factor for cardiovascular diseases. Also, higher tHcy levels have been linked to carotid atherosclerosis as assessed by IMT. However, controversies still exist regarding whether a slight increase in IMT represents focal atherosclerosis. Namely, IMT below certain levels could merely represent an adaptive response to the changes in shear stress, lumen diameter, tensile stress, and pressure. Given such controversies, we focused on atheromatous plaques as defined by focal increases in IMT ≥1.1 mm. This is in accordance with our prior study that showed the normal limit of IMT to be ≤1.0 mm. Additionally, we have demonstrated the validity of such a definition in a comparison with pathological findings.

In the present study, tHcy levels appeared to be lower than those reported for white populations. The tHcy levels may be determined by genetic and/or environmental factors, among which low dietary folate and vitamin B6 intake are important for inducing hyperhomocysteinemia. Compared with North America and Europe, vegetable consumption is relatively high and meat consumption is low in Japan, implying more intake of folate and vitamin B6. Thus, differences in the dietary habits could contribute to the lower tHcy levels found in this study.

Despite the lower tHcy levels, plaque score increased linearly with tHcy, suggesting an association between carotid atherosclerosis and tHcy levels. This finding is consistent with those of Selhub et al and Malinow et al, who have shown such associations even when tHcy levels are within normal ranges. Also, plaque score had a significant correlation with age and was higher in men than women and in smokers than nonsmokers, supporting associations between carotid atherosclerosis and such risk factors.

To further examine the link between carotid atherosclerosis and tHcy levels, multiple regression analysis was performed (Table 2). When traditional atherosclerotic risk factors were controlled for, tHcy was found to be significantly associated with plaque score, suggesting a potential effect of higher tHcy in the evolution of carotid atherosclerosis. This finding is consistent with a previous study showing associations between tHcy and carotid plaque area. Additionally, age and male sex were associated with plaque score independently of other risk factors, supporting previous studies. In the present study, hypertension, diabetes, hyperlipidemia, and smoking habits did not have independent associations with plaque score. Although we realize that duration and severity of such risk factors are important for the initiation and progression of atherosclerosis, we defined them as binary variables, which could have diluted their associations with plaque score. Also, the prevalence of diabetes and hyperlipidemia was relatively low, limiting our statistical power to examine their link with the plaque score.

As a manifestation of cerebral small-artery diseases, lacunar infarction is the most prevalent type of ischemic stroke in Japanese people. Although hypertension and diabetes mellitus are often found in such patients, no established atherosclerotic risk factors are present in 18% of them. In contrast to the well-defined association between tHcy and large artery atherosclerosis, the relationships between tHcy levels and small artery diseases are still controversial. FathBender et al have reported that homocysteine injures small perforating arteries rather than major cerebral arteries. Also, Evers et al reported that tHcy levels are higher in patients with lacunar infarction than in those with other stroke subtypes. In the present study, each 1-µmol/L-higher tHcy level was associated with a 1.37-fold-higher likelihood of lacunar infarction. Moreover, the association was modified little when age, sex, and other traditional atherosclerotic risk factors were controlled for. These results provide evidence for the association between higher tHcy level and prevalent lacunar infarction in Japanese people.

The present study has certain limitations. First, dietary habits such as meat, alcohol, and coffee consumption, which were not considered in this study, have been shown to affect tHcy levels. Second, tHcy levels in lacunar infarction patients could be underestimated because of lifestyle improvements after the infarction was diagnosed, potentially diluting the association between lacunar infarction and tHcy. Taken together, they indicate that larger prospective studies are necessary to establish whether higher tHcy levels serve as a harbinger for insidious carotid and cerebrovascular diseases.

In summary, plasma tHcy levels appear to be associated with carotid atherosclerotic plaques and lacunar infarction in the Japanese. Because tHcy levels are modifiable by lifestyle improvement, the modifications could have a potential therapeutic value for the prevention of carotid plaque formation and lacunar infarction.

**Acknowledgment**

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References


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