Low Concentration of Serum Total Cholesterol Is Associated With Multifocal Signal Loss Lesions on Gradient-Echo Magnetic Resonance Imaging

Analysis of Risk Factors for Multifocal Signal Loss Lesions

Seung-Hoon Lee, MD; Hee-Joon Bae, MD; Byung-Woo Yoon, MD, PhD; Ho Kim, PhD; Dong-Eog Kim, MD; Jae-Kyu Roh, MD, PhD

Background and Purpose—Multifocal signal loss lesions (MSLLs) on T2*-weighted gradient-echo (GE) MRI are believed to be microbleeds histopathologically. Previous epidemiological studies suggested that low serum cholesterol is associated with the increased risk of intracerebral hemorrhage. We investigated risk factors of MSLLs and the relationship between lipid profiles and MSLLs on GE-MRI.

Methods—We included consecutively 172 patients who underwent brain MRI. MSLLs on GE-MRI were counted by 2 neurologists separately and graded by consensus. The concentrations of lipid profiles were categorized as quartiles, and the MSLLs were graded as absent (total count, 0), mild (1 to 2), moderate (3 to 10), and severe (>10).

Results—The mean concentrations of total cholesterol and low-density lipoprotein cholesterol were significantly lower in patients with a severe degree of MSLLs than in those without MSLL (P<0.05). By multivariate analysis, MSLLs were significantly correlated with hypertension (odds ratio [OR], 3.42; 95% CI, 1.17 to 9.97), leukoaraiosis (OR, 4.62; 95% CI, 2.87 to 7.41), the lowest quartile of serum total cholesterol (4.27 mmol/L; OR, 10.91; 95% CI, 3.98 to 25.57), and the highest quartile of high-density lipoprotein (1.47 mmol/L; OR, 3.5; 95% CI, 1.45 to 8.29).

Conclusions—Our results suggest that both the lipid profile levels and the severity of hypertension may be closely associated with MSLLs on GE-MRI. (Stroke. 2002;33:2845-2849.)

Key Words: cholesterol ■ intracerebral hemorrhage ■ magnetic resonance imaging

T2*-weighted gradient-echo (GE) MRI is very sensitive in detecting small hemorrhages or calcifications via magnetic susceptibility effects. It has been reported that small multifocal signal loss lesions (MSLLs) on GE-MRI are frequently detected in patients with chronic hypertension or cerebral amyloid angiopathy. MSLLs were pathologically demonstrated as old, very small extravasations of blood, and as a result, causal relationships with intracerebral hemorrhage (ICH) have been suggested. Recently, we reported that hemorrhagic lacune is a predictor of ICH versus infarction in patients with no or mild leukoaraiosis. Generally, hemorrhagic stroke has been reported to occur at higher rates in persons with low levels of serum total cholesterol (TC), but this relationship remains controversial. However, because of the possibility of such a relationship, we thought that the possible association between serum TC level and MSLLs deserved to be investigated since there have been no studies to date on this topic. Thus, we undertook this study to delineate the association between MSLLs and lipid profiles and to determine the risk factors for MSLLs by GE-MRI. We hoped that such an investigation might give important information on the contentious association between lipid profiles and ICH.

Methods

Subjects

We prospectively evaluated 223 consecutive patients who required brain MRI studies because of neurological abnormalities at the Seoul National University Hospital, Department of Neurology, from March 1997 to July 1998. In consideration of possible alterations of lipid levels in acute stroke, we excluded patients who also had neurological symptom onset >24 hours before admission. As a result, the study population amounted to 172 subjects (100 men, 72 women). Their mean age was 64.1±10.1 years, and ages ranged from 37 to 92 years.

Clinical and Laboratory Findings

The patients underwent a careful medical history, a physical and neurological examination, ECG, echocardiography, and laboratory tests, which included lipid profiles. Diagnosis of major vascular risk...
Typical T2*-weighted GE-MRI shows mild (a; 1 to 2), moderate (b; 3 to 10), and severe (c; >10) grades of MSLLs. Although the MSLLs were counted in a whole brain area, a representative slice of each is displayed.

factors was based on history and laboratory findings. Hypertension was considered present if a subject had ≥2 of the following: (1) repeated blood pressure readings >160/95 mm Hg at intervals of ≥1 week, (2) a history of hypertension and/or antihypertensive medication, and (3) findings of target organ damage, including hypertensive retinopathy or left ventricular hypertrophy, on ECG or echocardiography. A diagnosis of diabetes mellitus was based on a history of diabetes mellitus with or without current treatment or fasting blood glucose levels >7.77 mmol/L (140 mg/dL). Glycosylated hemoglobin (hemoglobin A1c) levels were also measured to exclude the influence of stress-induced hyperglycemia (>8% values). A history of smoking was coded as present if a subject was a current smoker or an ex-smoker who had quit smoking within 5 years of admission.23

To measure lipid levels, venous blood was taken in each patient within 24 hours of admission after a 12-hour fast. Serum TC and triglycerides (TG) were analyzed with enzymatic colorimetric methods and reagents from Roche Diagnostics GmbH. High-density lipoprotein cholesterol (HDLC) was analyzed with homogeneous enzymatic colorimetric methods and reagents from Kyowa Medex Co. Ltd. Low-density lipoprotein cholesterol (LDLC) was calculated from the following equation: LDLC = TC - HDLC - 0.2 × TG.24

MRI Evaluation
MRI studies were performed with a 1.5-T superconducting magnet (Signa, GE Medical Systems). The standardized MRI protocol consisted of axial T2-weighted spin-echo (repetition time [TR], 2500 to 4500; echo time [TE], 80 to 112 ms; flip angle, 20°; slice thickness, 5 mm; gap width, 2 mm), axial T2*-weighted gradient-echo sequences (TR, 600 to 8000; TE, 26 ms; flip angle, 20°; slice thickness, 5 mm; gap width, 2 mm), and MR angiography (MRA; 3-dimensional time-of-flight sequence; TR/TE 30/2.7 ms; flip angle, 20°; field of view, 220 × 170 mm; matrix size, 256 × 192; number of excitations, 2; slice thickness, 1.4 mm; gap width, 0.7 mm). MSLLs were defined as homogeneous round signal loss lesions with a diameter up to 5 mm on GE-MRI. The number of MSLLs was counted in the whole brain area by 2 authors (S.-H. L. and H.-J. B.) separately and determined by consensus. The reviewers were blinded to clinical and demographic data. Lesions within the subarachnoid space and areas of symmetric hypointensity of the globus pallidus, likely to represent adjacent pial blood vessels and calcification, respectively, were not included. Because the numbers of MSLLs were not distributed normally (Kolmogorov-Smirnov test, P<0.001), we classified the degree as absent, mild (total number of MSLLs, 1 to 2), moderate (3 through 10), and severe (>10) (the Figure). On T2-weighted spin-echo images, leukoaraiosis was classified as absent, punctate, early confluent, or confluent abnormality according to the method proposed by Fazekas et al.23 MRA was used to document intracranial large-artery diseases, which were defined as >50% luminal narrowing in the intracranial internal carotid, anterior cerebral, middle cerebral, posterior cerebral, basilar, or intracranial vertebral arteries.

**Results**

The patient population consisted of 107 patients with acute stroke who were admitted within 1 day of ictus and 65 nonstroke patients with other neurological diseases. Subject characteristics are shown in Table 1. MSLLs on GE-MRI were found in 62 of the subjects (36.0%), and the degree of MSLLs was mild in 25 patients (40.3%), moderate in 21 patients (33.9%), and severe in 16 patients (25.8%); 25 patients (40.3%) had ICH, whereas 25 of 32 patients (78.1%) with ICH had MSLLs. Leukoaraiosis was found in 119 patients (69.9%); 69 (40.1%) had punctate, 36 (20.9%) had early confluent, and 14 (8.1%) had confluent abnormalities. Of 119 hypertensive patients, leukoaraiosis was found in 98

**Statistical Analysis**

Two sets of analyses were performed. First, 1-way ANOVA with Scheffé’s post hoc test was performed to compare the lipid profiles means in each group of MSLLs. For this analysis, TC and HDLC original data values were used, but HDLC and TG values were used after logarithmic transformation because they were not normally distributed (Kolmogorov-Smirnov test, P<0.05). In the second form of statistical analysis, we used the univariate and multivariate cumulative logits model26 to quantify the effects of risk factors on the MSLLs. The cumulative logits model is an extension of ordinary logistic regression analysis, which is used when the main response is a multilevel ordinal variable. In this model, 3 log odds ratios (ORs; absent versus others, absent or mild versus others, and absent, mild, or moderate versus severe) were defined and assumed to be parallel, which means that the effects of the risk factors are the same for the different log odds but that the intercepts are different. Two dummy variables were created for this analysis. These indicated <25th and >75th percentile for each lipid profile variable because of the nonlinear relationship between MSLLs and the lipid profile variables. Other variables, except for leukoaraiosis, were analyzed as dummy variables in the regression models. Leukoaraiosis was treated as a continuous variable (0=absent, 1=punctate, 2=early confluent, and 3=confluent) because a linear relationship was observed in the univariate logistic regression analysis. Proc logistic and proc catmod in SAS 6.12 were used for the cumulative logits models.
patients (82.4%), and large-artery disease was found in 64 patients (37.2%).

The distribution in serum lipid concentration according to MSLL grade and the cutoff values of lipid profiles in quartiles are presented in Table 2. In the 4 quartiles of TC, MSLLs were most frequently observed in the lowest quartile, whereas MSLLs were significantly lower than those of the absent group post hoc test: TC, P = 0.026; LDLC, P = 0.009; HDLC, P = 0.023). As a result of univariate analysis using the cumulative logits model, age, hypertension, cigarette smoking, TC, LDLC, and HDLC level also correlated positively with the degree of MSLL group significantly lower than those of the absent group (Scheffé’s post hoc test: TC, P = 0.019; LDLC, P = 0.009; Table 3). Specifically, the mean concentrations of both TC and LDLC in the severe MSLL group were significantly lower than those of the absent group (Scheffé’s post hoc test: TC, P = 0.019; LDLC, P = 0.009).

TABLE 2. Distribution of Levels of Lipid Profiles According to Grades of MSLL

<table>
<thead>
<tr>
<th>Grade of MSLL, n (rate)</th>
<th>Serum Concentration, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>Mild</td>
</tr>
<tr>
<td>TC &lt;25</td>
<td>TC &gt;25–4.94</td>
</tr>
<tr>
<td>25–50</td>
<td>LDLC &lt;2.38</td>
</tr>
<tr>
<td>51–75</td>
<td>HDLC &lt;0.98</td>
</tr>
<tr>
<td>&gt;75</td>
<td>TG &lt;0.90</td>
</tr>
</tbody>
</table>

Rate indicates fraction of each quartile.

Discussion

Our results indicate that very low TC levels are strongly related with increased numbers of MSLLs on GE-MRI, which is accepted as a risk factor of ICH. In addition, a high HDLC level also correlated positively with the degree of MSLLs. To the best of our knowledge, this is the first study to demonstrate relationships between lipid profiles and MSLLs.

This study reveals indirectly the influence of low serum TC on ICH using GE-MRI. MSLLs are closely related to ICH in 2 respects. First, MSLLs share histopathological findings with ICH. MSLLs visualized on GE-MRI are usually due to microbleeds, which are observed as focal hemosiderin deposits or organized miliary pseudoaneurysms accompanied with microbleeds, which are observed as focal hemosiderin deposits or organized miliary pseudoaneurysms accompanied with moderate to severe fibrohyalinosis of small arterioles. These findings signify minor previous extravasation of blood from fibrohyalinized vessels; thus, the MSLLs observed on GE-MRI may readily be explained as the resultant hemosid.

TABLE 3. Comparison of Lipid Profiles Among the Groups of MSLLs

<table>
<thead>
<tr>
<th>Grade of MSLL</th>
<th>TC, mmol/L</th>
<th>LDLC, mmol/L</th>
<th>HDLC, mmol/L</th>
<th>TG, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>4.97±0.98</td>
<td>3.02±0.86</td>
<td>1.24±0.36</td>
<td>1.57±0.87</td>
</tr>
<tr>
<td>Mild</td>
<td>4.78±1.09</td>
<td>2.85±0.95</td>
<td>1.38±0.49</td>
<td>1.19±0.73</td>
</tr>
<tr>
<td>Moderate</td>
<td>5.01±1.01</td>
<td>3.18±0.39</td>
<td>1.33±0.41</td>
<td>1.06±0.55</td>
</tr>
<tr>
<td>Severe</td>
<td>4.16±0.72</td>
<td>2.30±0.62</td>
<td>1.11±0.34</td>
<td>1.69±1.17</td>
</tr>
</tbody>
</table>

Data are mean±SD.

*One-way ANOVA test.
†Logarithmic transformation used for HDLC and TG.
erin deposits. Second, MSLLs are found more frequently in patients with ICH than in the patients without ICH. Because of these findings, MSLLs may deserve to be treated as a “silent ICH.” In addition, it is highly possible that MSLLs share vascular risk factors with ICH. MSLLs, however, are usually observed as multiple or multifocal lesions. Although this study used MSLLs instead of ICH, this method may show the effect of lipids on vascular rupture more sensitively.

Many large epidemiological studies have concluded that low serum TC might be a risk factor for ICH. A cohort study of Japanese men living in Hawaii and Japan showed a higher incidence of hemorrhagic stroke in those in the lowest quintile of serum TC (<4.86 mmol/L) with a relative risk of 2.55. Similarly, an inverse relationship was reported between serum TC level and the risk of death from hemorrhagic stroke. The present study shows that patients with low serum TC (<4.27 mmol/L) have a relative risk of 10.9 for an increased number of MSLLs. Therefore, we consider that the MSLLs on GE-MRI reflect the influence of low serum TC as arterial ICH. Parenchymal bleeding caused by rupture of a small vessel is a unique phenomenon of the brain and is rarely found in the heart. For this reason, relatively little information is available on the effect of high HDLC levels on small vessels. A case-control study by Lindgren et al showed significantly lower HDLC values in a subgroup of ICH, but the small sample size (12 patients with ICH) prevents generalization. Hence, we hope that the finding presented here, namely that a high concentration of HDLC is independently related to the degree of MSLLs, provides new information about the “dual effect” of HDLC that protects the cerebral vessels from ischemic insults but might offer a vulnerability to vascular rupture. We recommend that the influence of a high HDLC level on vascular weakening be intensively investigated.

In this study, we introduced a method of grading MSLLs. We did so because the variability with respect to the number of MSLLs was very wide and because we expected to observe a dose-response type of relationship between vascular risk factors and the MSLL degree. As a result, the lowest quartile of serum TC was very strongly correlated with the degree of MSLLs compared with results from previously reported ICH studies. We consider that this method would contribute to the enhancement of the sensitivity of results. However, our study has some limitations. First, LDLC levels were calculated with the Friedewald equation instead of being obtained by direct examination. In addition, the blood samples used to detail the lipid profiles of acute stroke patients were obtained only after ictus. Thus, we tried to minimize bias by strictly including only patients who arrived at our hospital within 24 hours of ictus in the case of stroke, but some possibility of selection bias exists. However, a prior study showed that no significant change was observed between the concentration of TC ≤48 hours after ictus and 3 months later in ICH patients, and the temporal changes in TC levels in acute cerebral infarction are not fully understood. From these results, the methods used in this study were considered to influence results only minimally.

Additionally, the present study reconfirmed that hypertension is a principal risk factor for MSLLs, as previously reported, and the grade of leukoaraiosis is well correlated with the grade of MSLL. Because of the various causes of leukoaraiosis, it is difficult to elucidate how leukoaraiosis is related to microbleeds in the cerebral vasculature. A recent report on the positive relation between left ventricular hypertrophy and leukoaraiosis suggests that leukoaraiosis may be associated with the severity or duration of hypertension. Therefore, our results indicate that the severity of hypertension may be associated with the occurrence of MSLLs. With regard to aging, Iribarren et al reported that the correlation between low serum TC and ICH was confined to elderly men ≥65 years; however, we did not observe this relationship, which suggests that the severity of hypertension rather than age may be the key factor in terms of vessel wall weakening.

In conclusion, this study shows that low serum TC and high HDLC may be risk factors for MSLLs, which have been known to be closely related to ICH, and that the presence of hypertension and the severity of leukoaraiosis are positively associated with the MSLL degree. Despite the large number

<table>
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<th>TABLE 4. Univariate Analysis</th>
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<tbody>
<tr>
<td>OR</td>
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<td>Age</td>
</tr>
<tr>
<td>Hypertension</td>
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<tr>
<td>DM</td>
</tr>
<tr>
<td>Smoking</td>
</tr>
<tr>
<td>Leukoaraiosis</td>
</tr>
<tr>
<td>Large-artery disease</td>
</tr>
<tr>
<td>TC &lt;25th percentile</td>
</tr>
<tr>
<td>TC &gt;75th percentile</td>
</tr>
<tr>
<td>LDLC &lt;25th percentile</td>
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<td>HDLC &gt;75th percentile</td>
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<tr>
<td>TG &lt;25th percentile</td>
</tr>
<tr>
<td>TG &gt;75th percentile</td>
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</tbody>
</table>

Cumulative logits model.

TABLE 5. Multivariate Analysis

| OR    | 95% CI  |
| Hypertension | 3.42 | 1.17–9.97 |
| Leukoaraiosis | 4.62 | 2.87–7.41 |
| TC <25th percentile | 10.91 | 3.98–25.57 |
| TC >75th percentile | 2.45 | 0.96–6.26 |
| HDLC <25th percentile | 0.92 | 0.37–2.33 |
| HDLC >75th percentile | 3.46 | 1.45–8.29 |

Cumulative logits model.
of epidemiological studies that have been undertaken on the relationship between TC and ICH, experimental studies on the pathomechanism are rare. Although decreased plasma fibrinogen activity\(^{31}\) and platelet aggregability\(^{32}\) resulting from low serum TC have been suggested, they remain to be proved. Further understanding of the pathophysiology is awaited.

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References

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