Objective  The aim of this study was to evaluate the relationship between fatty liver disease (FLD) and cerebrovascular disease.

Methods  We conducted a cross-sectional study of 76 consecutive healthy subjects who participated in a two-day hospitalized health checkup program. The maximal intima-media thickness (IMT) of the common carotid artery and bifurcation of the carotid artery as well as the plaque score (PS) were evaluated on carotid artery ultrasonography. Fluid attenuated inversion recovery brain MRI was used to determine the presence of asymptomatic cerebral lesions and periventricular hyperintensity. Brain MR angiography was used to evaluate the degree of intracranial main artery stenosis (ICAS). FLD was diagnosed based on the ultrasonographic pattern.

Results  The PS and IMT did not differ between the FLD (n=24) and non-FLD (n=52) groups. There was a tendency toward a higher percentage of multiple lacunar lesions in the non-FLD group than in the FLD group. ICAS was significantly more frequent in subjects in the FLD group than those in the non-FLD group (25.0% vs. 5.8%). A logistic regression analysis revealed that age and FLD were significant determinants of ICAS.

Conclusion  Our study findings suggest a significant association between ICAS and FLD.

Key words: fatty liver disease, intracranial main artery stenosis, carotid artery atherosclerosis, cerebral infarction

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talized health checkup program to evaluate the link between FLD and asymptomatic stroke.

Materials and Methods

A total of 76 consecutive subjects (61 men and 15 women; mean age ± standard deviation: 61.4 ± 8.0 years) with no previous history of symptomatic stroke or acute coronary syndrome participated in the two-day hospitalized health checkup program at Dokkyo Medical University Hospital were included in this study. Background factors, including age, sex, daily alcohol intake, smoking, body mass index (BMI) and medications for atherosclerotic diseases, such as diabetes mellitus, hypertension and dyslipidemia, were evaluated in all participants. Blood samples were obtained in an overnight fasting state, and the total cholesterol, triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), fasting plasma glucose and glycated hemoglobin [HbA1c (NGSP)] levels were measured. Among the subjects with a daily drinking habit, no patients reported alcohol consumption of 20 g or more per day. Patients who had not smoked for the past five years were defined as non-smokers. Hypertension was defined as a systolic blood pressure of ≥140 mmHg or a diastolic blood pressure of ≥90 mmHg (9). Diabetes mellitus was defined as a fasting plasma glucose level of ≥126 mg/dL, a casual plasma glucose level of ≥200 mg/dL or an HbA1c level of ≥6.5%. Dyslipidemia was defined as an LDL-C level of ≥140 mg/dL, an HDL-C level of <40 mg/dL or a TG level of ≥150 mg/dL in a fasting state (10). Patients receiving medications for hypertension, diabetes mellitus and/or dyslipidemia were defined as having each disease.

Brain magnetic resonance imaging (MRI) was performed on 1.5 T (Symphony, Sonata, Siemens Japan Company, Tokyo, Japan). Fluid-attenuated inversion recovery (FLAIR) images (TR=10,000 msec, TE=98 msec, IT=2,500 msec) were used to evaluate the degree of periventricular hyperintensity (PVH) and asymptomatic lacunar lesions in the deep white matter. PVH was classified into the following five grades (11): Grade 0, no PVH (normal); Grade 1, PVH on the edges of the anterior horn of the lateral ventricle in the basal ganglion; Grade 2, linear or strip-shaped changes with smooth margins in the body region or irregularly-shaped PVH observed in less than half of the white matter around the lateral ventricle; Grade 3, PVH observed in more than half of the white matter surrounding the lateral ventricle; and Grade 4, PVH diffusely distributed in the subcortical white matter. Asymptomatic lacunar lesions were defined as hyperintense lesions (<15 mm) with or without central hypointense areas on FLAIR images. Multiple lacunar lesions (MLL) were defined as the presence of two or more lacunar lesions on FLAIR images. Intracranial main artery stenosis (ICAS) was defined as stenosis of ≥50% in the main intracranial arteries based on the findings of MR angiography (12). Y.A. and K.S. evaluated the MRI and MR angiography findings. Abdominal ultrasound was performed by a gastroenterologist with a 3.5-4 MHz convex array transducer to detect the presence of hepatorenal contrast, increased intrathecal levels, deep attenuation and obscuration of the intrathecal vessels and/or bile ducts in order to assess the degree of FLD. The serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT) levels were measured in all subjects.

Carotid artery ultrasonography was performed by H.T., a board-certified fellow of the Japan Society of Ultrasonics in Medicine, using a linear probe (center frequency 6.5 MHz, SonoSite 180, FUJIFILM SonoSite, Tokyo, Japan) to determine the plaque score (PS) (13), maximal intima-media thickness of the common carotid artery (IMT-Cmax) and maximal intima-media thickness of the bifurcation of the carotid artery (IMT-Bmax).

This study was approved by the institutional review board of Dokkyo Medical University, and all study participants provided their informed consent.

Statistical analysis

The patients were classified into either the FLD group or non-FLD group, and background factors and the findings of brain MRI and carotid artery ultrasonography were compared between the groups using Mann-Whitney’s U test. Variables with a p value of <0.1 were included in the logistic regression analysis. Spearman’s rank correlation was used to determine the correlations between the serum biochemical data and atherosclerotic markers. A p value of <0.05 denoted the presence of a statistically significant difference. Examination findings predicting the presence of FLD were also analyzed. All statistical analyses were performed using the IBM SPSS Statics software package (version 21.0, Tokyo, Japan).

Results

The clinical background factors of the FLD (n=24) and non-FLD (n=52) groups are shown in Table 1. Hepatitis C virus (HCV) antibodies and hepatitis B surface (HBs) antibodies were found to be positive in three and one subject, respectively, in the non-FLD group only. The percentage of men tended to be higher and the BMI values were significantly higher in the FLD group than in the non-FLD group. The two groups did not differ with respect to the remaining background factors; however, the serum ALT levels were significantly higher in the FLD group than in the non-FLD group.

In contrast, the atherosclerotic indices obtained on carotid artery ultrasound, such as PS, IMT-Cmax and IMT-Bmax, did not differ between the FLD group and the non-FLD group. Regarding the brain MRI findings, a PVH of ≥2 was less frequently observed in the FLD group than in the non-FLD group (8.33% vs. 19.2%), although the difference was not statistically significant. There was a tendency toward a higher rate of MLL in the non-FLD group compared to that observed in the FLD group. As for the MRA find-
ings, ICAS was significantly more frequent in the subjects in the FLD group than in those in the non-FLD group (25.0% vs. 5.7%; Table 2).

A logistic regression analysis was performed to identify factors contributing to the presence of FLD, including gender, BMI, MLL and ICAS as independent variables. No correlations were found between FLD and gender. A high BMI (odds ratio (OR) 1.24, 95% confidence interval (CI) 1.03-1.51, p=0.0255), the absence of MLL (OR 0.272, 95%CI 0.0743-0.999, p=0.0499) and ICAS (OR 5.92, 95%CI 1.14-12.61, p=0.029) were found to be significant predictors of FLD.

On the other hand, the ICAS group showed a higher mean age and higher rates of daily alcohol intake, hypertension and FLD compared with the non-ICAS group (Table 4). The logistic regression analysis performed to predict the presence of ICAS, including the variables of age, smoking, hypertension and FLD, which revealed age (OR 1.24, 95%CI 1.06-1.15, p=0.00815) and FLD (OR 23.4, 95%CI 2.22-248, p=0.00876) to be significant determinants.

When dividing the patients based on the presence of MLL, the MLL group exhibited a higher mean age and higher rates of smoking and hypertension than the non-MLL group. FLD tended to be less frequent in the MLL group than in the non-MLL group (Table 4). The logistic regression analysis of MLL, including the variables of age, smoking, hypertension and FLD, identified age (OR 1.09, 95%CI 1.01-1.18, p=0.0294) and FLD (OR 0.265, 95%CI 0.0758-0.923, p=0.00946) to be significant predictors of MLL. In the FLD group, the serum AST and ALT levels were not correlated with IMT-Cmax: maximal intima/media thickness of the bifurcation of the carotid artery, IMT-Bmax: maximal intima/media thickness of the common carotid artery, MLL: multiple lacunar lesions, PVH: periventricular hyperintensity.

Table 1. Clinical Background Factors of the FLD and Non-FLD Groups

<table>
<thead>
<tr>
<th>Factor</th>
<th>FLD group</th>
<th>Non-FLD group</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>24</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>Age (median; range)</td>
<td>61.5, 47.81</td>
<td>61.0, 40-79</td>
<td>0.938</td>
</tr>
<tr>
<td>Male (%)</td>
<td>91.7</td>
<td>75.0</td>
<td>0.0919</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>50.0</td>
<td>42.3</td>
<td>0.533</td>
</tr>
<tr>
<td>Daily alcohol (%)</td>
<td>45.8</td>
<td>55.8</td>
<td>0.423</td>
</tr>
<tr>
<td>Medical treatment (%)</td>
<td>29.2</td>
<td>15.4</td>
<td>0.163</td>
</tr>
<tr>
<td>BMI (kg/m²; median; range)</td>
<td>25.8; 18.9-34.8</td>
<td>24.8; 18.6-30.4</td>
<td>0.0127</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>37.5</td>
<td>38.5</td>
<td>0.937</td>
</tr>
<tr>
<td>Dyslipidemia (%)</td>
<td>51.2</td>
<td>40.4</td>
<td>0.265</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>8.33</td>
<td>11.5</td>
<td>0.674</td>
</tr>
<tr>
<td>AST (median; range)</td>
<td>23.1; 9-72</td>
<td>18.6; 8-62</td>
<td>0.231</td>
</tr>
<tr>
<td>ALT (median; range)</td>
<td>33.1; 13-139</td>
<td>15.7; 5-38</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>GGT (median; range)</td>
<td>51; 17-220</td>
<td>42.2; 9-212</td>
<td>0.111</td>
</tr>
</tbody>
</table>

* Under medical treatment for vascular risk factors, such as hypertension, dyslipidemia and diabetes mellitus

Abbreviations: BMI: Body mass index, FLD: fatty liver disease, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT: gamma-glutamyl transpeptidase

Table 3. Clinical Background Factors of the ICAS and Non-ICAS Groups

<table>
<thead>
<tr>
<th>Factor</th>
<th>ICAS** group</th>
<th>Non-ICAS group</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>9</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Age (median; range)</td>
<td>72; 59-81</td>
<td>61; 40-79</td>
<td>0.008832</td>
</tr>
<tr>
<td>Male (%)</td>
<td>100</td>
<td>77.6</td>
<td>0.115</td>
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<tr>
<td>Smoking (%)</td>
<td>44.4</td>
<td>44.8</td>
<td>0.985</td>
</tr>
<tr>
<td>Daily Alcohol (%)</td>
<td>88.9</td>
<td>47.8</td>
<td>0.0212</td>
</tr>
<tr>
<td>Medical treatment (%)</td>
<td>33.3</td>
<td>17.9</td>
<td>0.278</td>
</tr>
<tr>
<td>BMI (kg/m²; median; range)</td>
<td>25.2; 21.9-29.0</td>
<td>25.0; 18.6-34.8</td>
<td>0.431</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>77.8</td>
<td>32.8</td>
<td>0.00963</td>
</tr>
<tr>
<td>Dyslipidemia (%)</td>
<td>44.4</td>
<td>44.8</td>
<td>0.985</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>0</td>
<td>11.9</td>
<td>0.276</td>
</tr>
<tr>
<td>Fatty liver (%)</td>
<td>66.7</td>
<td>26.9</td>
<td>0.0166</td>
</tr>
</tbody>
</table>

* Under medical treatment for vascular risk factors, such as hypertension, dyslipidemia and diabetes mellitus

** Intracranial artery stenosis ≥ 50% on magnetic resonance angiography
change from those of the original analysis including all subjects.

**Discussion**

In this study, we investigated the relationships between FLD and the presence of carotid artery lesions, intracranial artery stenosis and microvascular ischemic or hemorrhagic changes. The study results demonstrated that the subjects with FLD had an increased rate of intracranial stenosis than those without FLD. The presence of ICAS remained a significant predictor of FLD after accounting for clinical variables in a logistic regression model. The presence of NAFLD has been reported to be associated with an increased coronary atherosclerotic burden (14, 15) and is independent risk factor for diabetes mellitus (16). However, the effects of NAFLD on ischemic stroke, intracranial artery stenosis and carotid atherosclerosis remain unclear. In this regard, our findings exhibiting a significantly higher rate of ICAS in the FLD group than in the non-FLD group are thought to be of clinical significance.

Several studies have assessed the correlation between FLD and the presence of carotid atherosclerosis and/or ischemic stroke. Li et al. conducted a community-based study of 1,809 participants 45 years of age or older in order to investigate whether the liver fat content is associated with carotid artery IMT. Their results showed that the maximum IMT, average IMT and PS were strongly associated with the liver fat content, after adjusting for age, gender, smoking history, LDL-C level and metabolic syndrome, suggesting that the liver fat content is independently associated with the presence of carotid atherosclerosis (17). A recent systematic review analyzing 3,497 subjects (1,427 patients and 2,070 controls) showed a significant association between NAFLD and carotid IMT: an estimated increase of 13% in carotid IMT was observed among the NAFLD patients compared with the controls (18). In addition, the pulsatility index of the basilar artery and carotid IMT have been reported to be higher in patients with NAFLD than in control subjects (19). As an increased pulsatility index of the basilar artery may indicate the presence of intracranial artery atherosclerosis, their findings support our observations of an increased rate of ICAS in the FLD group; however, in our study, carotid atherosclerotic indices, such as the PS and IMT-Bmax and C-max, were not statistically different between the FLD and non-FLD groups. In a cross-sectional study using diffusion-weighted MRI, adult patients with suspected stroke were assigned to either the ischemic stroke group (n=103) or the control group (n=200) based on diffusion-weighted MRI findings. The authors found that the serum alanine aminotransferase and aspartate aminotransferase concentrations were significantly elevated in the ischemic stroke group compared with those observed in the control group (7). This finding remained significant even after controlling for stroke risk factors, indicating that NAFLD is an independent risk factor for ischemic stroke. Among 1,221 Japanese men and women recruited from a health checkup program, the incidence of cardiovascular disease, including ischemic stroke, was higher in the 231 subjects with NAFLD than in the 990 subjects without NAFLD (8). In the present study, although the rate of MLL was higher in the non-FLD group, the logistic regression model showed a weak association between MLL and FLD. However, considering the finding that ICAS was significantly more frequent in the FLD group than in the non-FLD group, further longitudinal studies with a large sample size may reveal a positive relationship between the presence of asymptomatic lacunar lesions and FLD.

In the FLD group, we found a weak but significant positive correlation between the serum GGT levels and the IMT-Cmax values. Kozakova et al. (20) found a significant relationship between FLD and carotid plaque, and serum GGT was identified to be an independent determinant of early carotid atherosclerosis. However, in their study, serum GGT was found to be an independent determinant of the presence of plaque in the carotid bulb, but not the arterial wall thick-
ness in the common carotid artery, which is slightly different from the present results. Although this difference may be due to differences in the study samples and design, among patients with FLD, the serum GGT levels may be a significant determinant of atherosclerotic changes. In addition, the serum GGT level may be considered a vascular risk biomarker related to oxidative stress (21).

Higher BMI values were found to be associated with FLD in this study, which may reflect the presence of increased visceral fat accumulation in patients with FLD. Hamaguchi et al. (8) showed NAFLD to be a strong predictor of cardiovascular disease, irrespective of the presence or absence of metabolic syndrome. Likewise, in the present study, the significant correlation between ICAS and FLD remained after accounting for BMI in the logistic regression model.

Because liver biopsies were not performed in this study, patients with NASH and simple steatosis may have been included. The significant association observed between ICAS and FLD may be attributed to the presence of NASH rather than simple steatosis, given that the study by Sanyal et al. (22) demonstrated the presence of oxidative stress and ultrastructural mitochondrial abnormalities, which are known to be risk factors for vascular disease and atherosclerosis, in patients with NASH compared with those with simple steatosis (23, 24).

One limitation of this study is the reliance on self-reported medication and alcohol use. Therefore, detailed information regarding medications (statins or angiotensin II receptor blockers) was not available, and alcoholic liver diseases may have been missed. We also cannot exclude the presence of liver diseases due to other causes, as no liver biopsies were performed. Second, our study included a small sample size, and the diagnosis of diabetes was made based on the results of single blood tests. Third, in this study, we evaluated only asymptomatic participants of a two-day hospitalized health checkup program, not symptomatic patients with stroke.

In conclusion, our study findings suggest a significant association between ICAS and FLD. Further longitudinal studies with large sample sizes are needed to confirm whether the presence of FLD is a risk factor for ICAS.

The authors state that they have no Conflict of Interest (COI).

Acknowledgement

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References

