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Lipoprotein Abnormalities in the Pathogenesis of Cerebral Infarction and Transient Ischemic Attack

ATSUSHI MURAI, M.D., TOMOJI TANAKA, M.D., TADAO MIYAHARA, M.D., AND MASAKUNI KAMEYAMA, M.D.

SUMMARY HDL- and LDL-cholesterol levels were determined by a heparin-Ca precipitation method in 89 survivors of cerebral infarction (CI) (75 males, 14 females) and 14 patients with transient ischemic attacks (TIA) (8 males, 6 females). The mean values of HDL-cholesterol concentration and HDL:LDL-cholesterol ratio for both sexes of CI patients were significantly lower than those of the healthy controls (37 males, 14 females). These values for CI patients were significantly lower than in patients with various diseases excluding cardiovascular disease, hepatic disease, hyperlipidemia, diabetes mellitus and degenerative disorders of the nervous system (46 males, 43 females). In patients with TIA, these differences were statistically significant only for men. Based on the patient's history, clinical signs and symptoms and the findings of computerized tomography and 4-vessel angiography, male CI patients were divided into 2 sub-groups, CI believed to be in the distribution of a perforating artery and CI in the distribution of a cortical artery; it was found that the HDL-cholesterol level and HDL:LDL-cholesterol ratio were significantly lower in the cortical artery group than in the perforating artery group, suggesting that these lipoprotein abnormalities may play a part in the pathogenesis of CI, particularly of the cortical artery area infarction.

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CONFLICTING REPORTS have been published about the significance of hyperlipidemia in the development of cerebrovascular disease. Some investigators, who have examined survivors of attacks of ischemic cerebrovascular disease, have observed elevated mean concentrations of serum cholesterol and/or triglyceride (TG),14-16 while others found no increase in serum lipid levels.17 In contrast, epidemiological studies conducted in several areas of Japan indicated that the incidence of cerebral infarction was inversely related to the mean level of serum cholesterol;18,19 ischemic cerebrovascular disease was found to be associated with a low serum cholesterol level in Japan, where the mean value of serum cholesterol has rarely exceeded 200 mg/dl.

Prospective cohort studies should provide the most reliable information on blood lipids as a risk factor. In the Framingham study, an association of blood lipid with the development of atherothrombotic cerebral infarction under age 60 was statistically significant only when associated with the development of atherothrombotic cerebral infarction. Regardless of the associated lipoprotein pattern, the risk of infarction increased in proportion to the serum cholesterol level, but pre-beta lipoprotein levels were unrelated to the risk when associated cholesterol levels were taken into account.20 Two prospective studies in Japan, one conducted in Hisayama21 and the other in Akabane and Asahi,22
found no significant relation between serum cholesterol and the development of cerebral infarction. It has been widely accepted that the serum cholesterol level is not associated with the development of cerebrovascular disease in Japan.

A growing body of evidence from epidemiological and biochemical studies suggests that high density lipoprotein (HDL) may protect against coronary heart disease.18-19 Lower HDL-cholesterol levels in patients with ischemic cerebrovascular disease were recently verified by Rössner et al.20-21 and Taggart et al.22 It is important to point out that the mean serum cholesterol levels of the normal subjects in these studies were near 250 mg/dl, which is much higher than in Japanese normal subjects. The Japanese are prone not to coronary heart disease but to cerebrovascular disease so it was believed desirable to learn more about the lipoprotein profile characteristic of the Japanese survivors of stroke.

Two types of arterial lesions are known to be responsible for cerebral infarction.23-27 One is atherosclerosis, which affects the large extra- and intracranial arteries and their main branches over the convexity of the brain. These are defined as cortical arteries in this study. The other type of lesion is fibrinoid necrosis, which affects small arteries penetrating the deep structures of the brain, such as the lenticulostriate, pontine and intracerebral small arteries. These are designated as perforating arteries in this study. It is postulated that arterial hypertension has a greater impact on perforating arteries while lipoprotein abnormalities exert a greater effect on cortical arteries.

In this study, survivors of ischemic cerebrovascular disease are classified into 2 sub-groups according to the presumed sites of arterial lesions, and lipoprotein abnormalities were characterized for each sub-group to assess their role in the pathogenesis of stroke.

**Subjects and Methods**

**Subjects**

Patients with stroke had survived cerebral infarction or a transient ischemic attack. The diagnosis of CI or TIA was confirmed by a history, clinical signs and symptoms, and computerized cerebral axial tomography (CT). Patients for whom the stroke was complicated by ischemic heart disease, peripheral arterial disease, hepatic disease, hyperlipidemia, diabetes mellitus or degenerative disorders of the central nervous system were not included.

The CI group was made up of 75 males and 14 females. The TIA group, composed of patients with lacunar stroke24 whose CT scans showed a small infarct of low density in the area of the basal ganglia which was believed responsible for the stroke. Patients with large cortical and/or subcortical infarct on CT scan were included in the cortical artery group. In patients where both findings were observed on the CT scan, the patient was assigned to the cortical artery group. Among the 75 male patients with CI, 18 patients who did not fall into either of these categories underwent 4-vessel angiography. Thirteen patients who showed significant atherosclerotic changes were assigned to the cortical artery group, and the remaining 5 patients who showed no significant atherosclerotic changes were assigned to the perforating artery group.

The patients in the perforating artery group comprised 38 males and 9 females. The cortical artery group was made up of 37 males and 5 females.

Two control groups were set up as follows:

- **Control Group I.** This consisted of a healthy group of 37 males and 14 females.
- **Control Group II.** This was a group composed of 46 male and 43 female in- and out-patients without ischemic heart disease, peripheral arterial disease, hepatic disease, hyperlipidemia, diabetes mellitus or degenerative disorders of the central nervous system.

**Methods**

Blood samples were collected by venipuncture after an overnight fast. Serum lipoproteins were fractionated by the modified heparin-calcium differential precipitation method developed by Noma et al.28 HDL was separated from the other lipoproteins by filtration after 30-min incubation with 0.015% (w/v) heparin and 35 mM CaCl₂. HDL plus LDL were fractionated after removal of VLDL-heparin complexes, which were formed at higher ionic strength, with anion-exchange resin. Cholesterol levels in the serum and the lipoprotein fractions, and triglyceride levels in the serum were determined by enzymatic methods.

In deciding whether or not a patient was hypertensive, the patient's history and blood pressure readings were considered. When these were not available, the presence of hypertension was judged by serial readings after the acute stage. Hypertension was defined as blood pressure above 160/95 mm Hg. Statistical analysis used the Student's t-test.

**Results**

The mean value of each variable was judged by comparing the stroke group with the 2 control groups. The age of the subjects in control group II was similar to the age of the 4 groups of patients with cerebrovascular disease: the CI group, perforating artery group, cortical artery group, and TIA group (table 1). Although control group II was made up of patients, the mean values of HDL-cholesterol concentration were similar to the mean values of the healthy control group I (table 2).
**LIPOPROTEIN ABNORMALITIES IN CI AND TIA/Murai et al.**

**TABLE 1. Age Distribution in Patients and Controls (Mean ± SD)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Age (years) (range)</td>
</tr>
<tr>
<td>Control Group I</td>
<td>37</td>
<td>36.4 ± 12.3 (20 - 61)</td>
</tr>
<tr>
<td>Control Group II</td>
<td>46</td>
<td>60.5 ± 13.7 (25 - 83)</td>
</tr>
<tr>
<td>Cerebral Infarction</td>
<td>75</td>
<td>62.2 ± 9.9 (30 - 80)</td>
</tr>
<tr>
<td>Cortical Artery Group</td>
<td>37</td>
<td>60.8 ± 11.5 (30 - 80)</td>
</tr>
<tr>
<td>Perforating Artery Group</td>
<td>38</td>
<td>63.6 ± 7.8 (49 - 79)</td>
</tr>
<tr>
<td>TIA</td>
<td>8</td>
<td>59.6 ± 14.0 (37 - 73)</td>
</tr>
</tbody>
</table>

**TABLE 2. Cholesterol Concentration of Serum Lipoproteins and HDL:LDL Cholesterol Ratio in Stroke Patients and Controls (Mean ± SEM)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Male Cholesterol concentration (mg/dl)</th>
<th>HDL:LDL cholesterol ratio</th>
<th>Female Cholesterol concentration (mg/dl)</th>
<th>HDL:LDL cholesterol ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>HDL</td>
<td>LDL</td>
<td>n</td>
</tr>
<tr>
<td>Control</td>
<td>37</td>
<td>181.2</td>
<td>53.2</td>
<td>121.7</td>
</tr>
<tr>
<td>Group I</td>
<td>± 5.2</td>
<td>± 2.2</td>
<td>± 4.8</td>
<td>± 0.023</td>
</tr>
<tr>
<td>Control</td>
<td>46</td>
<td>187.8</td>
<td>52.5</td>
<td>126.1</td>
</tr>
<tr>
<td>Group II</td>
<td>± 5.1</td>
<td>± 1.9</td>
<td>± 4.7</td>
<td>± 0.024</td>
</tr>
<tr>
<td>Cerebral Infarction</td>
<td>75</td>
<td>197.2</td>
<td>41.6</td>
<td>146.3</td>
</tr>
<tr>
<td></td>
<td>± 5.7</td>
<td>± 1.1</td>
<td>± 5.4</td>
<td>± 0.013</td>
</tr>
<tr>
<td>TIA</td>
<td>8</td>
<td>183.9</td>
<td>40.4</td>
<td>134.6</td>
</tr>
<tr>
<td></td>
<td>± 10.9</td>
<td>± 2.2</td>
<td>± 10.5</td>
<td>± 0.026</td>
</tr>
</tbody>
</table>

*(versus control group I) and * (versus control group II). Significant differences are indicated (Student's t-test); * or , p < 0.05 ** or , p < 0.01 *** or , p < 0.001.

**Lipoprotein Profile in Patients with CI or TIA**

1) **HDL-cholesterol** (table 2). The mean value of HDL-cholesterol levels in males was 53.2 mg/dl in control group I and 52.5 mg/dl in control group II; the value in females was 60.0 mg/dl in control group I and 59.1 mg/dl in control group II. The mean value for the CI group was 41.6 mg/dl in males and 46.1 mg/dl in females. These values were significantly lower than those for either control group for both sexes. The mean value for the male patients with TIA was also significantly lower than that of either control group. This difference, however, was not significant when the female TIA group was compared with the control groups.

2) **LDL-cholesterol** (table 2). For comparison of LDL-cholesterol levels, control group II was believed more suitable because of age-related changes in LDL-cholesterol levels. In male patients, the mean value of LDL-cholesterol level for the CI group was significantly higher than that of the control group.

3) **HDL:LDL-cholesterol ratio** (table 2). The mean values of the male CI and TIA groups were 0.307 and 0.309 respectively, and were significantly lower than those for either control group I or II. In females, the values for the patients with CI or TIA were also lower, but the difference between the patients with TIA and control group II was not statistically significant.

**Differences Between Divided Sub-groups**

Based on the presumed site of the arterial lesion, patients with cerebral infarction were divided into two sub-groups (table 3).

1) **HDL- and LDL-Cholesterol and HDL:LDL-Cholesterol Ratio** (table 3). In males, the mean values for the HDL-cholesterol level and HDL:LDL-cholesterol ratio were significantly lower in the "cortical artery group" as compared to those in the "perforating artery group." The mean level of LDL-cholesterol was significantly higher in the former group than in the latter. In females, similar changes were observed, but only the difference in HDL:LDL-cholesterol ratio was statistically significant.

2) **Serum Cholesterol** (tables 2 and 3). When the "cortical artery group," "perforating artery group,"
TABLE 3 Serum Lipoprotein Profiles and Incidence of Hypertension in Two Subgroups of Stroke Patients (Mean ± SEM)

<table>
<thead>
<tr>
<th>Group</th>
<th>Cholesterol concentration (mg/dl) Total</th>
<th>HDL</th>
<th>LDL</th>
<th>Male HDL/LDL-cholesterol ratio</th>
<th>serum TG (mg/dl)</th>
<th>No. of hypertensive (%)</th>
<th>Cholesterol concentration (mg/dl) Total</th>
<th>HDL</th>
<th>LDL</th>
<th>Female HDL/LDL-cholesterol ratio</th>
<th>serum TG (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortical Artery</td>
<td>208.4 ± 9.0*</td>
<td>39.1</td>
<td>160.4</td>
<td>0.264 ± 0.015</td>
<td>(27.0)</td>
<td>155</td>
<td>10</td>
<td>242.4 ± 31.1</td>
<td>39.2</td>
<td>185.2</td>
<td>0.231</td>
</tr>
<tr>
<td>Perforating Artery</td>
<td>186.2 ± 6.4</td>
<td>44.1</td>
<td>132.6</td>
<td>0.348 ± 0.018</td>
<td>(56.8)</td>
<td>136</td>
<td>21</td>
<td>207.5 ± 13.3</td>
<td>50.0</td>
<td>144.6</td>
<td>0.356</td>
</tr>
</tbody>
</table>

* (versus control group I), + (versus control group II), ** (versus perforating artery group). Significant differences are indicated (Student's t-test): * or + or ++: p < 0.05, ** or ++ or +++: p < 0.01; *** or +++ or ++++: p < 0.001.

TIA group, control group I and control group II were compared in males, the mean value of serum cholesterol levels in the "cortical artery group" was significantly higher than that in the 4 other groups. A considerable number of patients in the "perforating artery group" had serum cholesterol levels under 170 mg/dl. In females, there were similar differences but they were not significant when compared to control group II.

3) Serum Triglyceride (table 3, fig. 1). Serum triglyceride concentrations tended to be higher in the "cortical artery group" than in the "perforating artery group."

4) Hypertension (table 3). The prevalence of arterial hypertension was 56.8% in the "perforating artery group" and 27.0% in the "cortical artery group." The difference between these 2 groups was statistically significant.

5) Age (fig. 2). For males, the HDL-cholesterol concentration was plotted against the age of patients. There was no consistent correlation between them in either the "cortical or perforating artery groups." This finding suggests that lowered HDL-cholesterol levels may be found irrespective of the age of patients, at least for male patients under age 80.

Discussion

Classification of patients with stroke has provided information important in evaluating the significance of an elevated serum cholesterol concentration. The mean value of serum cholesterol in the "cortical artery group..."
group" was slightly higher but still within normal limits, when compared to control group II (208.4 mg/dl and 187.8 mg/dl), and the mean value for the "perforating artery group" was almost the same as that of the control group II (186.2 mg/dl and 187.8 mg/dl). In studies of survivors of ischemic cerebrovascular disease, it is likely that patients with CI in the distribution of a cortical artery were included in excess, leading to an undue elevation of the mean serum cholesterol concentration. Our results are compatible with the finding that the level of serum cholesterol is not related to cerebral infarction in prospective studies. This does not necessarily exclude the possibility that certain types of hyperlipoproteinemia can accelerate the development of CI even in Japanese.

Whether or not HDL-cholesterol levels in apparently healthy subjects change with advancing age remains controversial. HDL-cholesterol levels appear to remain virtually constant after maturation, although for both sexes they have been reported to decrease after the sixth decade. In the present study, the HDL-cholesterol level of the younger control group I was nearly equal to that of older control group II, indicating that the HDL-cholesterol level was not affected by the age of these patients.

Robinson et al. first reported the significantly reduced level of alpha-lipoprotein cholesterol in patients with cerebral thrombosis, but this finding was limited to patients under the age of 60. Rössner et al., studying patients with stroke under age 55, found reduced levels of HDL-cholesterol as compared to normal controls, but in Noma's study where patients with stroke with a mean age of 73.5 and 70.0 in females were examined, no significant decrease of HDL-cholesterol concentration was found. Thus, the age of the patient seems an important determining factor for the level of HDL-cholesterol. In this study there was no significant correlation between the HDL-cholesterol level and the age of patients with stroke, although all patients were male and under 80 years of age. Since HDL-cholesterol levels for adult females are undoubtedly higher than for adult males, Omata's study, in which the results for male and female patients were evaluated as one group, is misleading.

In the present study, male and female patients with TIA were examined separately and reduced levels of HDL-cholesterol for both sexes were found, although the reduction was significant only for men, results compatible with a recent report by Sirtori et al.

Since the respective actions of HDL and LDL on atherogenesis are believed to be in opposition, it is possible that the HDL:LDL-cholesterol ratio may serve as a more sensitive predictor of atherosclerotic vascular disease than either of the lipoprotein cholesterol levels used singly. This possibility is supported by the present study, in which this ratio appeared to be a better discriminator when the TIA group was compared with controls, or when the "cortical artery group" was compared with the "perforating artery group."

Arterial hypertension is believed to be the strongest risk factor for stroke and to exert a greater effect on the perforating arteries than on the cortical arteries. Kameyama pointed out in his pathohistological study that the incidence of small and medium-sized cerebral infarctions increased in proportion to the degree of hypertension, and the incidence of large infarction was independent of the severity of hypertension. These results suggest that hypertension is not the cause of vessel change in the major cerebral arteries but is in small cerebral arteries. Some factor(s) other than hypertension may contribute to atherothrombotic occlusion of the major cerebral arteries. In his study, blood pressure levels measured before the onset of stroke were later used to evaluate the effect of these levels on the subsequent development of stroke. Mathew et al., who studied patients with occlusive cerebrovascular disease using cerebral angiography, found that the patient group with only intracranial small-vessel lesions had about twice the prevalence of hypertension than the patient group with intracranial major-vessel lesions or extracranial arterial lesions. The group with small-vessel lesions showed a lower level of serum cholesterol and triglyceride than the group with large vessel lesions. The small-vessel lesion group in their study roughly corresponded to the "perforating artery group" and the large-vessel lesion group to the "cortical artery group."

The two main types of arterial lesions underlying cerebral infarction are atherosclerosis and fibrinoid necrosis. In the present study, attempts were made to clinically divide the patients with stroke, as clearly as possible, into 2 sub-groups: the "cortical artery group," in which atherosclerosis was believed responsible for the infarctions, and the "perforating artery group," in which fibrinoid necrosis was believed responsible. A large cortical and/or subcortical low density area is typical of an infarction in the distribution of a cortical artery; this is usually associated with atherothrombotic occlusion of this artery. Certain findings on CT scan are considered suggestive of narrowing of a cortical artery: a small infarct situated in the watershed area and a small infarct in the area of the basal ganglia later followed by asymmetric ipsilateral atrophic changes of the brain. Neurological signs and symptoms such as aphasia, homonymous hemianopia and cortical blindness are also useful in detecting the cortical artery occlusion. An accurate assignment of patients to the "perforating artery group" is hampered by considerable difficulty in distinguishing an infarct produced by thrombotic occlusion of a perforating artery itself from an infarct produced by embolic occlusion of a perforating artery. The embolus may come from an atherothrombotic lesion far from the perforating artery occluded and the resulting infarction in the distribution of the perforating artery would mean that the patient should be assigned to the "cortical artery group." In the present study most patients in the "perforating artery group" were diagnosed as having lacunar stroke, which is not uncommon in Japan. It is believed that the cause of the lacune is probably thrombosis, but the possibility of an embolus can not be excluded. Thirteen patients in the "cortical artery group" and 5 in the...
"perforating artery group" had 4-vessel angiography. No significant atherosclerotic changes were found in any of the patients in the latter group. In addition, the incidence of hypertension was significantly higher in the "perforating artery group" than in the "cortical artery group," which is consistent with the findings of Mathew's study.28 We propose our diagnostic procedure as a useful means of classifying patients with stroke and assessing the role of lipoprotein abnormalities in the development of ischemic cerebrovascular disease.

References

Lipoprotein abnormalities in the pathogenesis of cerebral infarction and transient ischemic attack.

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