Cerebral Atherosclerosis in Japanese

Part 4: Relationship Between Lipid Content and Macroscopic Severity of Atherosclerosis

MOTOOMI NAKAMURA, M.D., KATSUMI IMAIZUMI, PH.D., YUTAKA KIKUCHI, M.D., AND HIDEO KANAIDE, M.D.

SUMMARY  In order to evaluate chemically the macroscopic scoring methods for severity of atherosclerosis in the cerebral arteries, concentrations of total lipids, esterified and free cholesterol and lipid phosphorus were compared to the macroscopic severity of lesions in the cerebral arteries obtained from 376 Japanese persons after unexpected death. An increase of cholesterol content was correlated significantly with an increase of Baker’s score and/or Gore’s atherosclerotic index. The correlation coefficient between Baker’s score and total or esterified cholesterol was the highest among the tested correlations ($r = 0.82, n = 376$).

Introduction

PREVIOUSLY we demonstrated that macroscopic severity as well as total cholesterol of the aorta and coronary arteries in Japanese autopsied cases were considerably lower when compared with those of Caucasians reported by others, and yet macroscopic severity of atherosclerosis of cerebral arteries from Japanese appeared to be equal or slightly greater than that reported in Caucasians.14

A slightly greater involvement of atherosclerosis of the cerebral arteries in Japanese compared to Caucasians also has been observed by others.4–6 Meyer et al. reported a significantly greater involvement of aortic and coronary atherosclerosis in South African Caucasians than in Bantus, but no difference in severity of cerebral atherosclerosis between South African Caucasians and Bantus.7,8 In order to clarify why ethnic difference in macroscopic severity of cerebral atherosclerosis is small, we studied chemical compositions of various arteries and found that lipids and glycosaminoglycans of the cerebral arteries were different from those of the aorta and coronary arteries.9,10

There are very few studies on the cerebral arteries evaluating the relationships between lipid content and macroscopic severity and also concerning the age-related changes of lipids. The present study concerns measurements of lipid content and macroscopic severity of atherosclerosis in cerebral arteries obtained from 376 cases of unexpected and sudden death in Japanese persons.

Methods

The 376 intracranial cerebral arteries were obtained at random from 717 cerebral arteries of cases autopsied for legal reasons, in which cause of death and macroscopic severity of atherosclerosis of the cerebral artery were reported previously.1 The cerebral arteries obtained at autopsy at the Tokyo Medical Examiner’s office were scored and stored in a deep freezer until chemical analysis of lipids was performed.

Scoring

The methods of Baker11 and of Gore and Tejada12 were used in grading the macroscopic severity of atherosclerotic changes in the cerebral arteries.

Measurement of Lipids

The individual cerebral arteries were cut into small pieces, added to 20 volumes of Folch’s mixture (chloroform: methanol = 2:1, v/v) and kept for 24 hours at room temperature with occasional shaking. This extraction was repeated twice. The extracts were combined and mixed with 0.2 volume of distilled water. The upper water phase was removed and the lower chloroform fraction was evaporated to dryness with a rotary evaporator. The residue was dissolved in chloroform-methanol (2:1, v/v). Total cholesterol (TC), free cholesterol (FC), esterified cholesterol (EC) and lipid phosphorus (LP) were determined as reported previously.13 The fat-free tissues were further treated with acetone and ether, and were desiccated to constant weight. The contents of lipids were expressed as milligram per gram of dried fat-free tissues.

Results and Discussion

The severity of atherosclerosis of 376 Japanese cerebral arteries, evaluated as Baker’s score and/or Gore’s atherosclerotic index (A.I.), was determined at each decade and according to sex as shown in tables 1 and 2. The atherosclerotic severity evaluated as Baker’s score or Gore’s A.I. at each decade was quite similar to that in the previous report dealing with 717 cases.1 An increase of Baker’s score and/or Gore’s A.I. with age was prominent in the fifth and seventh decade in men and in the sixth and eighth decade in women as shown in the previous study.1

The amount of TC, EC, FC, and LP tended to increase with age. EC increased from 2.5 in the second decade to 18.2 mg per gram defatted dried tissue in the seventh decade. The increase of EC with an increase of age was higher than that of other lipids. An increase of EC or FC as well as Baker’s score or Gore’s A.I. was most prominent in the fifth and seventh decade in men and the sixth and eighth decade in women.

The amount of TC, EC, and FC as well as macroscopic severity in the fifth decade was significantly higher in men than in women. However, there was no remarkable difference in LP between men and women. An increase of TC, EC or FC with age was parallel to that of macroscopic severity of atherosclerosis. Table 3 demonstrates the correlation between age, Gore’s A.I., Baker’s score, and lipid content of the arteries. All correlations were highly significant ($p < 0.01$). The highest positive correlation ($r = 0.82$)
Table 1: Macroscopic Severity of Atherosclerosis and Lipid Content of Japanese Cerebral Arteries

<table>
<thead>
<tr>
<th>Decade</th>
<th>No. of cases</th>
<th>Gore's A.I.</th>
<th>Baker's score</th>
<th>Total cholesterol</th>
<th>Free cholesterol</th>
<th>Esterified cholesterol</th>
<th>Lipid-phosphorus</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>4</td>
<td>0.20 ± 0.27</td>
<td>0.11 ± 0.13</td>
<td>0.20 ± 0.05</td>
<td>2.2 ± 0.28</td>
<td>1.7 ± 0.13</td>
<td>0.99 ± 0.05</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>0.69 ± 0.48</td>
<td>0.27 ± 0.05</td>
<td>0.28 ± 0.02</td>
<td>3.1 ± 0.58</td>
<td>1.9 ± 0.28</td>
<td>1.19 ± 0.06</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>1.11 ± 0.63</td>
<td>0.30 ± 0.09</td>
<td>1.24 ± 0.22</td>
<td>5.0 ± 1.3</td>
<td>2.5 ± 0.33</td>
<td>1.34 ± 0.04</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>1.11 ± 0.54</td>
<td>0.33 ± 0.10</td>
<td>1.24 ± 0.22</td>
<td>5.0 ± 1.3</td>
<td>2.5 ± 0.33</td>
<td>1.34 ± 0.04</td>
</tr>
<tr>
<td>6</td>
<td>15</td>
<td>1.11 ± 0.54</td>
<td>0.33 ± 0.10</td>
<td>1.24 ± 0.22</td>
<td>5.0 ± 1.3</td>
<td>2.5 ± 0.33</td>
<td>1.34 ± 0.04</td>
</tr>
<tr>
<td>7</td>
<td>17</td>
<td>1.11 ± 0.54</td>
<td>0.33 ± 0.10</td>
<td>1.24 ± 0.22</td>
<td>5.0 ± 1.3</td>
<td>2.5 ± 0.33</td>
<td>1.34 ± 0.04</td>
</tr>
<tr>
<td>8</td>
<td>18</td>
<td>1.11 ± 0.54</td>
<td>0.33 ± 0.10</td>
<td>1.24 ± 0.22</td>
<td>5.0 ± 1.3</td>
<td>2.5 ± 0.33</td>
<td>1.34 ± 0.04</td>
</tr>
</tbody>
</table>

Values represent mean ± SE.
Statistical comparison was made between men and women of corresponding age groups (*p < 0.01, †p < 0.05).

Lipid content was expressed as milligram per gram fat-free dried tissues.

Figure 1. Fitted polynomial curve expressing the relationship between Baker's score and total cholesterol.
CEREBRAL ATHEROSCLEROSIS IN JAPANESE. PART 4/Nakamura et al.

TABLE 2  Progression of Macroscopic Severity and Lipid Content of Cerebral Arteries With Age

<table>
<thead>
<tr>
<th>Decade</th>
<th>No. of cases</th>
<th>Gore's A.I.</th>
<th>Baker's score</th>
<th>Total cholesterol</th>
<th>Free cholesterol</th>
<th>Esterified cholesterol</th>
<th>Lipid-phosphorus</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>7</td>
<td>0.17 ± 0.02</td>
<td>2.1 ± 0.4</td>
<td>6.5 ± 0.3</td>
<td>3.5 ± 0.6</td>
<td>0.5 ± 0.3</td>
<td>0.86 ± 0.09</td>
</tr>
<tr>
<td>3</td>
<td>36</td>
<td>0.27 ± 0.04</td>
<td>2.9 ± 0.8</td>
<td>7.4 ± 0.7</td>
<td>3.6 ± 0.7</td>
<td>0.7 ± 0.7</td>
<td>0.44 ± 0.04</td>
</tr>
<tr>
<td>4</td>
<td>69</td>
<td>0.34 ± 0.05</td>
<td>3.7 ± 1.1</td>
<td>9.2 ± 1.3</td>
<td>4.5 ± 1.1</td>
<td>1.1 ± 0.7</td>
<td>0.04 ± 0.04</td>
</tr>
<tr>
<td>5</td>
<td>55</td>
<td>0.92 ± 0.23</td>
<td>10.6 ± 1.4</td>
<td>13.7 ± 1.6</td>
<td>6.8 ± 1.4</td>
<td>1.1 ± 0.7</td>
<td>0.04 ± 0.04</td>
</tr>
<tr>
<td>6</td>
<td>70</td>
<td>1.26 ± 0.20</td>
<td>12.9 ± 1.4</td>
<td>18.6 ± 2.2</td>
<td>10.7 ± 1.4</td>
<td>1.6 ± 1.4</td>
<td>0.04 ± 0.04</td>
</tr>
<tr>
<td>7</td>
<td>93</td>
<td>4.11 ± 0.58</td>
<td>24.1 ± 2.8</td>
<td>37.4 ± 3.0</td>
<td>19.2 ± 2.5</td>
<td>2.2 ± 1.8</td>
<td>0.13 ± 0.04</td>
</tr>
<tr>
<td>8</td>
<td>34</td>
<td>5.93 ± 0.97</td>
<td>29.8 ± 3.1</td>
<td>54.9 ± 4.9</td>
<td>25.9 ± 2.9</td>
<td>3.0 ± 1.8</td>
<td>0.14 ± 0.04</td>
</tr>
<tr>
<td>9</td>
<td>12</td>
<td>6.56 ± 1.48</td>
<td>31.4 ± 5.5</td>
<td>50.2 ± 9.7</td>
<td>24.9 ± 7.4</td>
<td>3.9 ± 9.7</td>
<td>0.14 ± 0.04</td>
</tr>
</tbody>
</table>

See footnote of table 1.

was found between Baker's score and EC or TC. Figure 1 shows the fitted polynomial curve, which was computed by least squares, expressing the relationship between Baker's score and TC. This approximation, \( Y = 10.76 - 1.93x + 0.138x^2 - 0.0011x^3 \) (\( Y = \text{TC}; \ x = \text{Baker's score} \)), fitted well with the individual values. Thus macroscopic severity, especially Baker's method for scoring the severity of cerebral atherosclerosis, relates closely to the extent of cholesterol deposition.

It would be difficult to compare the present results on the progression of cerebral atherosclerosis with age in Japanese cases with that of other races, because of different techniques of analysis employed. However, it would be valuable to compare our data on the increase of lipid content and/or macroscopic severity with that of others. The relationship of Gore's A.I. or TC and age in the present study is compared with that of South African Caucasians and Bantus by Meyer et al. and of U.S. (St. Louis) Caucasians by Liang and Kirk\(^3\) in figures 2 and 3. As shown in figures 2 and 3, changes of TC with age were similar to those of Gore's A.I. in both Japanese and South African Caucasians or Bantus. In Japanese cases, changes of TC and/or Gore's A.I. with age were described by the following fitted polynomials, \( Y = 6.4 - 0.15x + 0.004x^2 \) (\( Y = \text{TC}; \ x = \text{age} \)) and \( Y = 3.00 - 0.232x + 0.0048x^2 + 0.00002x^3 \) (\( Y = \text{Gore's A.I.;} \ x = \text{age} \)), respectively.

The amount of TC increased from 6.5, 9.0, 9.1 and 7.1 in the second decade to 19.6, 21.7, 16.1 and 21.8 mg per gram of sample.

FIGURE 2. Age-related changes of Gore's A.I. of cerebral arteries from Japanese, South African Caucasians and Bantus.

FIGURE 3. Age-related changes of total cholesterol content of cerebral arteries from Japanese, South African Caucasians, Bantus and U.S. (St. Louis) Caucasians. Meyer et al. originally expressed cholesterol content as gram percent of the absolute dry material.\(^8\) Liang and Kirk originally expressed cholesterol content as percent of wet weight.\(^9\)
dried tissue weight in the sixth decade in Japanese, South
African Caucasians or Bantus and U.S. (St. Louis)
Caucasians, respectively. Although there seemed to be no
significant difference in increase of Gore's A.I. and/or TC
with age between the four races from the second to the sixth
decade, atherosclerotic severity in terms of not only
macroscopic grading but also cholesterol content appeared
to be slightly greater in Japanese cases in the seventh decade
than in Caucasians. However, the number of cases of
Caucasians was limited compared to ours, and there were no
data beyond the eighth decade. Further studies will be re-
quired to clarify the differences of severity of cerebral
atherosclerosis among different races.

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San Francisco, for his invaluable help in the preparation of this report.

Cerebral Atherosclerosis in Japanese

Part 5: Relationship Between Cholesterol Deposition and
Glycosaminoglycans

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YASUHIDE NAKASHIMA, M.D., YUTAKA KIKUCHI, M.D., AND KENZO TANAKA, M.D.*

SUMMARY Concentrations of various lipids and glycosaminogly-
cans (GAG) in the intima of the grossly normal and ather-
sclerotic cerebral arteries were compared with those of the aorta
and coronary arteries. The lowest percentage of esterified cholesterol
(4C) in total cholesterol, and of chondroitin sulfate-4/6 (CS-4/6) in
total glycosaminoglycans and the highest percentage of heparin sul-
force (HS) in total GAG are the characteristic features of the normal
intima of normal cerebral arteries when compared with those in the
aorta and coronary artery.

In the cerebral arterial intimas, but not in the aorta or coronary
arteries, there was a significant positive correlation between contents
of C and percentage and total content of CS-4/6. Atherogenesis in
cerebral arteries is discussed in comparison to that of the aorta and
 coronary vessels.

Introduction

SEVERAL STUDIES of changes of lipids and acid glycosaminoglycans (GAG) in human aortas with increasing
age or increased atherosclerosis have been reported.1,2 With

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TABLE 3. The Correlation Coefficients (r) of Age, Gore's A.I., and Baker's Score Versus Contents of Total Cholesterol, Free Cholesterol, Esterified Cholesterol, and Lipid-Phosphorus (576 Cases)

<table>
<thead>
<tr>
<th>Lipids</th>
<th>Age</th>
<th>Gore's A.I.</th>
<th>Baker's score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>0.53</td>
<td>0.75</td>
<td>0.82</td>
</tr>
<tr>
<td>Free cholesterol</td>
<td>0.48</td>
<td>0.70</td>
<td>0.76</td>
</tr>
<tr>
<td>Esterified cholesterol</td>
<td>0.53</td>
<td>0.74</td>
<td>0.82</td>
</tr>
<tr>
<td>Lipid-phosphorus</td>
<td>0.45</td>
<td>0.60</td>
<td>0.73</td>
</tr>
</tbody>
</table>
M Nakamura, K Imaizumi, Y Kikuchi and H Kanaide

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