Correlations Between Serum Cholesterol and Vascular Lesions in Fabry Disease Patients

Hiroki Katsuta, MD; Kazuya Tsuboi, MD, PhD; Hiroshi Yamamoto, MD, PhD; Hiromi Goto, MD, PhD

Background: Fabry disease is an X-linked lysosomal storage disorder and shows globotriosylceramide (Gb3) accumulation in multiple organs, resulting from a deficiency of α-galactosidase. In patients with Fabry disease, cardiovascular disease occurs at an early age. Previous studies have shown that serum levels of high-density lipoprotein-cholesterol (HDL-C) increase in this disease, yet its clinical significance for cardiovascular disease remains unclear.

Methods and Results: In order to determine why the serum HDL-cholesterol is high in various cardiovascular diseases of Fabry disease patients, we evaluated the serum lipid profiles, ocular vascular lesions, and levels of serum vascular endothelial growth factor (VEGF) and intercellular adhesion molecule-1 in 69 patients with Fabry disease diagnosed by genetic examination. The serum HDL-C/total cholesterol (T-Chol) ratio was significantly high, especially in male patients (41.5±1.7%) regardless of body mass index. Ocular vascular lesions were more likely to occur in female patients with a high HDL-C/T-Chol ratio compared with most male patients. Female patients with a high HDL-C/T-Chol ratio also presented a high serum VEGF level, suggesting that vascular endothelium dysfunction and arteriosclerotic changes progress more severely than in patients with a normal HDL-C/T-Chol ratio. In most patients, enzyme replacement therapy improved serum Gb3 and lyso-Gb3 levels, but these Gb3 and lyso-Gb3 still remained higher than in healthy controls, which appears to result in continuous vascular arteriosclerotic changes.

Conclusions: We concluded that increased low-density lipoprotein-cholesterol uptake to the vascular wall caused by endothelial dysfunction is likely to contribute to the high HDL-C/T-Chol ratio observed in Fabry disease patients.

Key Words: Enzyme replacement therapy; Fabry disease; High-density lipoprotein-cholesterol; Microvascular lesions; Sphingolipidosis
adhesion molecule-1 (ICAM-1), because serum VEGF and ICAM-1 are useful for detecting the early stages of atherosclerotic lesions and are thought to be related to diabetic microangiopathy. In previous studies, these cytokines were higher in this disease than in healthy controls. We also measured serum Gb3 and lyso-Gb3 levels before and after ERT based on our hypothesis that the cholesterol profile is affected by these factors.

### Methods

We carried out an observational study of Fabry disease patients at Nagoya Central Hospital who were confirmed by clinical manifestation and genetic testing. The mutation of E66Q is now thought to be a genetic polymorphism, so this mutation was excluded. Patients with no test results for HDL-C and low-density lipoprotein-cholesterol (LDL-C) were also excluded. The sex proportions in this study were 30 males and 37 females. The study was conducted in accordance with the Declaration of Helsinki and applicable local laws and regulations. Patients provided written informed consent before inclusion in the study.

In previous studies, serum cholesterol levels have shown no obvious changes before and after ERT, so we used the blood test results from the first visit to hospital regardless of ERT. The serum lipid profile was determined by enzymatic method. The reference ranges were: total cholesterol (T-Chol): 100–200 mg/dL in men and 70–150 mg/dL in women; LDL-C: 30–149 mg/dL; triglycerides (TG): 40–150 mg/dL; HDL-C: 40–85 mg/dL in men and 50–90 mg/dL in women; and the HDL-C/T-Chol (or LDL-C) ratio was 25–35%.

In male and female patients, respectively, the serum lipid profiles were as follows: T-Chol, 166.8±5.2 mg/dL (4.32±0.13 mmol/L) and 203.8±5.4 mg/dL (5.28±0.14 mmol/L), which was almost the same as in previous studies of Fabry disease patients. In female patients, TG, 77.1±6.4 mg/dL (0.87±0.07 mmol/L) and 105.4±10.4 mg/dL (1.19±0.12 mmol/L); LDL-C, 87.5±4.8 mg/dL (2.27±0.12 mmol/L) and 119.0±4.4 mg/dL (3.08±0.11 mmol/L); HDL-C, 67.8±2.3 mg/dL (1.74±0.12 mmol/L) and 70.2±2.0 mg/dL (1.78±0.11 mmol/L). A total of 10 female patients had dyslipidemia; all showed only high LDL-C.

The HDL-C/T-Chol ratio was higher than the reference range in 22 of the 30 male patients and in 20 of the 37 female patients. The HDL-C/T-Chol ratio was 41.5±1.7% in males and 35.2±1.2% in females. We also analyzed whether this cholesterol profile correlated with age, whether this cholesterol profile correlated with age, but did not find a clear correlation in either sex. Male patients aged >50 years were also included in the high HDL-C/T-Chol ratio group, but they had significant heart atrophy and renal dysfunction, which suggested that a high HDL-C/T-Chol ratio does not contribute to cardiovascular protection.

We also analyzed the relationship between BMI and the HDL-C/T-Chol ratio. As shown in Figure 1 the correlation coefficient was −0.097 in males and −0.231 in females. However, aneurysms of the conjunctival vessels were much fewer (4 of 61 patients) compared with previous studies reporting that aneurysms were observed in 68–97% of patients.

### Ocular Vascular Lesions and Relationship to Serum Cholesterol

Abnormal findings in the ocular vessels were identified in 23 of the 27 male patients and in 17 of 34 female patients. Common vascular lesions were arteriolar tortuosity, arteriolar narrowing, broadening of the light reflex with minimal arteriovenous compression in fundic vessels. Conjunctival microaneurysms, vessel overswellling or irregularities of the vessel’s caliber were found in 4 patients. The ocular vascular lesions of the patients in this study are shown in Table 2. A previous study reported tortuosity of retinal vessels, venous vascular aneurysmal dilatation and caliber irregularities. However, aneurysms of the conjunctival vessels were much fewer (4 of 61 patients) compared with previous studies reporting that aneurysms were observed in 68–97% patients.

<table>
<thead>
<tr>
<th>Patients’ Characteristics</th>
<th>Male (n=30)</th>
<th>Female (n=37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>31.4±4.1 (6–55)</td>
<td>45.67±4.8 (14–64)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>20.1±1.2</td>
<td>21.5±1.0</td>
</tr>
<tr>
<td>T-Chol (mg/dL)</td>
<td>130–220</td>
<td>166.8±5.2</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>65–139</td>
<td>87.5±4.8</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>40–150</td>
<td>77.1±6.4</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>40–85 (M)/40–95 (F)</td>
<td>67.8±2.3</td>
</tr>
<tr>
<td>HDL/Total (%)</td>
<td>25–35</td>
<td>41.5±1.7*</td>
</tr>
</tbody>
</table>

*P<0.05. BMI, body mass index; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; T-Chol, total cholesterol.
levels change after ERT, we compared 14 patients with serum VEGF and ICAM-1 data before and after ERT using the Wilcoxon rank sum test. Neither VEGF nor ICAM showed a decrease after ERT, so we used the serum VEGF and ICAM-1 data from the first hospital visit regardless of administration of ERT. The serum VEGF level was >38.3 pg/mL in 12 of 18 male patients and in 12 of 22 female patients. ICAM-1 was higher than the reference range in 16 of 18 male patients and in 13 of 22 female patients. We analyzed the correlation between these cytokines and the HDL-C/T-Chol ratio. Serum VEGF was significantly higher in patients with a high HDL-C/T-Chol ratio, especially in female patients (P=0.138 in males; P=0.047 in females) (Figure 2). Serum ICAM-1 did not show a clear correlation with the HDL-C/T-Chol ratio. Previous studies have shown that serum VEGF and ICAM-1 levels increase from an early stage of atherosclerosis. So it is likely that a high HDL-C/T-Chol ratio reflects some vascular lesions in Fabry disease. In addition, patients with ocular vascular lesions had more tendency towards a high serum VEGF level. Our results suggested that a high HDL-C/T-Chol ratio and high serum VEGF indicate

Table 2. Ocular Vascular Lesions in Fabry Disease Patients

<table>
<thead>
<tr>
<th>Vascular lesion</th>
<th>Male (%)</th>
<th>Female (%)</th>
</tr>
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<tbody>
<tr>
<td>Fundic vascular tortuosity</td>
<td>20 (74)</td>
<td>8 (24)</td>
</tr>
<tr>
<td>Scheie classification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H0</td>
<td>11 (41)</td>
<td>21 (62)</td>
</tr>
<tr>
<td>H1</td>
<td>13 (48)</td>
<td>11 (32)</td>
</tr>
<tr>
<td>H2</td>
<td>3 (11)</td>
<td>2 (6)</td>
</tr>
<tr>
<td>H3</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>S0</td>
<td>10 (37)</td>
<td>21 (62)</td>
</tr>
<tr>
<td>S1</td>
<td>15 (56)</td>
<td>12 (35)</td>
</tr>
<tr>
<td>S2</td>
<td>1 (4)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>S3</td>
<td>1 (4)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

In addition to these vascular lesions, conjunctival microaneurysms (1 patient), vessel overswelling (1 patient) and irregularities of the vessel caliber (2 patients) were observed.

Table 3. Relationship Between Ocular Vascular Lesions and HDL-C/T-Chol Level

<table>
<thead>
<tr>
<th>HDL-C/T-Chol</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;35% (%)</td>
<td>17 (85)</td>
<td>7 (100)</td>
</tr>
<tr>
<td>≤35% (%)</td>
<td>3 (15)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

The results of Fisher’s exact test were P=0.55 in males and P=0.17 in females. HDL-C, high-density lipoprotein-cholesterol; T-Chol, total cholesterol.

In this study, only 3 male and 4 female patients showed hypertension and only 10 female patients showed dyslipidemia; hypertensive and arteriosclerotic changes occurred in most of the male patients and half of the female patients, suggesting that Fabry disease, but not the more common diseases such as hypertension and dyslipidemia, causes arteriolar lesions.

We also analyzed the correlation between the HDL-C/T-Chol ratio and ocular vascular lesions (Table 3). We divided our patients into 2 groups according to the HDL-C/T-Chol ratio (≤35% vs. >35%). In male patients, ocular vascular lesions were found at almost the same rate in both groups. However, female patients with a high HDL-C/T-Chol ratio (>35%) were more likely to have ocular vascular lesions, although not statistically significant in Fisher’s exact test. Our results suggested that a high HDL-C level does not play a vascular protective role in Fabry disease patients.

Serum VEGF and ICAM-1 Levels and Relationship to Serum Cholesterol

In order to determine whether serum VEGF and ICAM-1 levels change after ERT, we compared 14 patients with serum VEGF and ICAM-1 data before and after ERT using the Wilcoxon rank sum test. Neither VEGF nor ICAM showed a decrease after ERT, so we used the serum VEGF and ICAM-1 data from the first hospital visit regardless of administration of ERT. The serum VEGF level was >38.3 pg/mL in 12 of 18 male patients and in 12 of 22 female patients. ICAM-1 was higher than the reference range in 16 of 18 male patients and in 13 of 22 female patients. We analyzed the correlation between these cytokines and the HDL-C/T-Chol ratio. Serum VEGF was significantly higher in patients with a high HDL-C/T-Chol ratio, especially in female patients (P=0.138 in males; P=0.047 in females) (Figure 2). Serum ICAM-1 did not show a clear correlation with the HDL-C/T-Chol ratio. Previous studies have shown that serum VEGF and ICAM-1 levels increase from an early stage of atherosclerosis. So it is likely that a high HDL-C/T-Chol ratio reflects some vascular lesions in Fabry disease. In addition, patients with ocular vascular lesions had more tendency towards a high serum VEGF level. Our results suggested that a high HDL-C/T-Chol ratio and high serum VEGF indicate
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Discussion

In this study, we analyzed the correlations between serum cholesterol levels and vascular lesions in Fabry disease patients. Our results showed that these patients had a high HDL-C/T-Chol ratio, vascular hypertensive and arteriosclerotic changes of the fundus and high serum VEGF and ICAM-1 levels. In the study by Cartwright et al, the average of serum HDL-C concentration in male patients was 1.46 mmol/L and 1.93 mmol/L in female patients. Stepień et al also reported almost the same cholesterol levels. In our results, the male HDL-C level was 1.74 mmol/L, which was higher than in the 2 previous studies. So the high HDL-C level in our study is consistent and unlikely to be affected by ethnic differences.

Ocular vascular lesions and increased VEGF levels were observed more frequently in patients with a high HDL-C/T-Chol ratio, especially female patients, suggesting that the HDL-C/T-Chol ratio is related to vascular lesions. This high HDL-C/T-Chol ratio was observed from an early age and did not change even after ERT, which is consistent with previous research. Other studies have shown that accumulated Gb3 in vascular endothelial cells induces vascular damage in Fabry disease patients.

Serum Gb3 and Lyso-Gb3 Levels

In order to determine why the HDL-C/T-Chol ratio and VEGF levels remain high, we analyzed the serum Gb3 and lyso-Gb3 levels before and after ERT (Table 4). For the data from “after ERT”, we used the latest blood test results from all patients who underwent this treatment for at least 3 months. In male patients, Gb3 decreased from 15.7 ng/mL to 6.49 ng/mL and lyso-Gb3 decreased from 83.4 pg/mL to 32.3 pg/mL on average. In female patients, Gb3 decreased from 9.00 ng/mL to 5.61 ng/mL and lyso-Gb3 decreased from 10.5 pg/mL to 6.84 pg/mL on average. These results suggested that ERT can decrease Gb3 and lyso-Gb3 levels. However, the reference range for Gb3 is 4.6±2.0 ng/mL and that of lyso-Gb3 is <1.2±0.1 pg/mL, so the serum levels were still higher in the present study patients than in healthy people, even after ERT. Based on our results, it is possible that high Gb3 and lyso-Gb3 levels may affect the continually high HDL-C/T-Chol ratio, and thus vascular lesions, even after ERT.

Table 4. Comparison of Serum Gb3 and Lyso-Gb3 Levels Before ERT and in the Latest Blood Test Results

<table>
<thead>
<tr>
<th></th>
<th>Threshold</th>
<th>Before ERT (n=16)</th>
<th>Latest (n=21)</th>
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</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gb3 concen. (ng/mL)</td>
<td>4.6±2.0</td>
<td>15.7 (8.6–25.6)</td>
<td>6.5 (6.0–11.0)</td>
</tr>
<tr>
<td>Lyso-Gb3 concen. (pg/mL)</td>
<td>&lt;1.2±0.1</td>
<td>83.4 (21.3–156.0)</td>
<td>32.3 (26.7–40.3)</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gb3 concen. (ng/mL)</td>
<td>4.6±2.0</td>
<td>9.0 (5.5–11.3)</td>
<td>5.6 (4.5–10.2)</td>
</tr>
<tr>
<td>Lyso-Gb3 concen. (pg/mL)</td>
<td>&lt;1.2±0.1</td>
<td>10.5 (6.7–14.2)</td>
<td>6.8 (4.9–9.5)</td>
</tr>
</tbody>
</table>

ERT, enzyme replacement therapy; Gb3, globotriosylceramide.
oxidative stress, reduces NO production and upregulates adhesion molecules and LDL-receptor expression. Moreover, K, expressed in vascular endothelial cells, is downregulated by Gb3-receptor expression. Based on these results, it is possible that a high HDL-C/T-Chol ratio is caused by increased endocytosis of LDL-C to the endothelial cells. Our results indicated that this endothelial dysfunction can start when young, even in heterozygote female patients. Considering that female patients with a high HDL-C/T-Chol ratio were more likely to show a high serum VEGF level, a high HDL-C/T-Chol ratio might reflect vascular endothelium dysfunction and arteriosclerotic changes, resulting in a more severe disease process.

Although the majority of patients in this study did not show hypertension, S1 and H1 in the Scheie classification and vascular tortuosity were commonly found, even in some young patients. Previous in vivo study using GLA knockout mice showed that lyso-Gb3 is a strong proliferation factor for vascular smooth muscle cells. Ocular vascular changes appear to reflect vascular wall thickening and narrowed vessel caliber. Other studies report increased basilar artery diameter in Fabry disease patients. In our study, serum lyso-Gb3 showed a significant decrease after ERT, but remained much higher than in healthy people, suggesting that present ERT protocols are not effective enough because of high lyso-Gb3 levels. In fact, other ways of evaluating vascular lesions and endothelial functions, such as IMT and FMD, have not shown outstanding improvement after ERT.

Recent in vitro studies suggested other mechanisms of vascular damage caused by this disease. In GLA-silencing cells, lipid raft domains show a significant increase. Because LDL-C is essential for lipid raft formation, LDL utilization might be increased in Fabry disease patients, which is likely to result in a relative increase of serum HDL-C. Moreover, the vascular tortuosity observed in this disease is likely to cause turbulent blood flow, which may contribute to the formation of focally distributed incipient athersclerotic lesions.

It is known that other lysosomal storage disorders are associated with HDL-C decrease. In Gaucher disease, this low HDL-C improves with ERT towards the normal range. In Niemann-Pick A, B and C disease, the HDL-C level is also low. One possible reason is the contribution of liver function. HDL-C is not only produced in peripheral tissues but also re-secreted by the liver. As with many other organs, liver function is affected in most lysosomal storage diseases, but not in Fabry disease, which may contribute to the high serum HDL-C level in this disease. However, further study is necessary to clarify more why HDL-C increases only in Fabry disease.

The limitation of this study is that the number of patients was small, especially those who had serum VEGF, ICAM-1, Gb3 and lyso-Gb3 levels measured. In addition, the relationships between the serum cholesterol profile and the risk of stroke, acute coronary syndrome etc. need to be further studied. By evaluating ocular vascular lesions or the serum VEGF level, we may be able to follow-up vascular damage. Despite these limitations, we believe our study sheds new light on Fabry disease, especially in relation to vascular damage.

Grants
No grants are provided in this study, but Kazuya Tsuboi receives consulting fee from Sumitomo Dainippon Pharma Co., Ltd.

References
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Supplementary Files

Supplementary File 1

Figure S1. Correlation between age and HDL/T-Chol ratio in Fabry disease patients.

Please find supplementary file(s):