Terminal stage cardiac findings in patients with cardiac Fabry disease: An electrocardiographic, echocardiographic, and autopsy study

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KEYWORDS
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Pathology;
Cardiomyopathies;
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Summary
Objectives: Fabry disease is caused by deficiency of α-galactosidase A, and typically causes multi-organ dysfunction. Patients with manifestations limited to the heart, mainly left ventricular hypertrophy (LVH), have been reported as a disease variation. We have reported a 3% prevalence of this cardiac variant in men with LVH, which we designated 'cardiac Fabry disease'. The purposes of this study were to evaluate the terminal stage cardiac manifestations and autopsy findings in patients with cardiac Fabry disease.

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Methods: We examined seven terminal stage patients with cardiac Fabry disease. During hospitalization, standard 12-lead electrocardiograms, Holter electrocardiograms, and echocardiograms were obtained. Autopsies were performed and performed along with microscopic findings were evaluated.

Results: Six patients died of heart failure and one of ventricular fibrillation. Electrocardiograms revealed the presence of conduction abnormalities and nonsustained ventricular tachycardia. Echocardiograms and autopsy findings revealed LVH in all patients. Localized basal posterior wall thinning of the left ventricle was detected in the six patients who died of heart failure. All patients had severe left ventricular dysfunction. Histologically, myocardial cells, but not cardiac vascular endothelial cells, showed glycosphingolipid accumulation. No accumulation was observed in other organs or in systemic vascular endothelial cells.

Conclusions: Severe left ventricular dysfunction with associated conduction disturbances and ventricular arrhythmias occur in patients with terminal stage cardiac Fabry disease. Furthermore, LVH is present and associated with thinning of the base of the left ventricular posterior wall. In contrast to typical Fabry disease, accumulation of glycosphingolipids was observed in myocardial cells but not in other organs.

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Introduction

Fabry disease is an X-linked recessive disorder caused by mutation of the gene encoding α-galactosidase A (α-gal A), one of the lysosomal hydrolases [1]. In this disease, glycosphingolipids, primarily globotriaosylceramide, accumulate progressively in lysosomes of vascular endothelial cells and smooth muscle cells, as well as in various cells in multiple organs, particularly the skin, kidneys, and heart [1]. Male patients with typical Fabry disease show almost complete absence of α-gal A activity. They exhibit symptoms of angiokeratoma, acroparesthesia, hypohidrosis, and corneal opacities that develop in early childhood and eventually die from complications affecting the kidney, brain, and/or heart in their fourth or fifth decade of life [1]. With respect to cardiac manifestations, left ventricular hypertrophy (LVH), valvular abnormalities, and electrocardiographic abnormalities, including arrhythmias, have been reported in many patients [2–19].

Male patients with an atypical form of Fabry disease with manifestations confined to the heart have been described [20–24]. These patients with this cardiac variant of Fabry disease have residual α-gal A activity, and the main manifestation is LVH induced by excessive accumulation of glycosphingolipid in myocardial cells [1]. We have reported that the cardiac variant of Fabry disease was detected in 7 of 230 male patients (about 3%) with LVH [25]. All of those patients presented with LVH as the main finding, with the plasma α-gal A activity remaining at 4–14% of the normal activity and lacking the symptoms observed in typical Fabry disease, such as angiokeratoma, acroparesthesia, hypohidrosis, and corneal opacity [25]. We designated this form of Fabry disease as ‘cardiac Fabry disease’ and proposed that it represents a new clinical entity [26]. There have been few detailed reports on the histologic findings of the heart and other organs in patients with cardiac Fabry disease [20,21,23]. The purposes of this study were to characterize the terminal stage cardiac manifestations and histologic findings of the heart and other organs in autopsied patients with cardiac Fabry disease.

Subjects and methods

Patients studied

Among nine consecutive male patients with cardiac Fabry disease who died between March 1992 and August 2005 during hospitalization at our institution, seven autopsied patients were examined. The diagnosis of cardiac Fabry disease was based on a low level of plasma α-gal A activity, the presence of LVH, and the absence of clinical manifestations of typical Fabry disease such as angiokeratoma, acroparesthesia, hypohidrosis, and corneal opacity. Two of the seven patients (Patients 5 and 6) received enzyme replacement therapy.

Electrocardiography and echocardiography

During hospitalization, standard 12-lead electrocardiograms (ECG), Holter ECG, and echocardiograms were obtained in all patients. The rhythm, heart rate, PR interval, QRS duration, and SV1 + RV5 amplitude were evaluated in the standard 12-lead
ECG. A PR interval ≥ 0.21 s and a QRS duration ≥ 0.12 s were considered as prolonged [27]. An abnormal Q-wave was defined as being 0.04 s or longer in duration and ≥1/4 of the R-wave amplitude in leads other than aVR. An SV1 + RV5 value ≥ 4.0 mV was considered indicative of the presence of left ventricular high voltage. In addition, Holter ECG was performed to evaluate the type and severity of arrhythmias.

The interventricular septal wall thickness, left ventricular posterior wall thickness, left atrial dimension, left ventricular end-diasstolic dimension, and left ventricular end-systolic dimension were evaluated by M-mode echocardiography. An interventricular septal wall or left ventricular posterior wall thickness of ≥ 13 mm was considered diagnostic of hypertrophy [28]. A left atrial dimension of ≥ 41 mm and a left ventricular end-diastolic dimension of ≥ 58 mm were considered to indicate chamber enlargement [29]. A left ventricular fractional shortening of ≤ 33% was considered abnormal [29]. The presence or absence and degree of abnormal left ventricular wall motion were evaluated visually [30].

**Histologic studies**

After obtaining written informed consent from family members, autopsies were performed. The heart, kidneys, skin, liver, lung, spleen, pancreas, and adrenal gland were obtained from six of the seven patients (Patients 1—4, 6, and 7) for histologic examination. Heart and kidneys only were obtained from Patient 5 for histologic evaluation.

Heart tissue from each patient was fixed in 10% buffered formalin, and the sections were stained with hematoxylin and eosin (H&E). In addition, cardiac specimens from five of the seven patients (Patients 1—4, and 7) were examined by osmium toluidine blue (TB) staining and electron microscopy. TB staining was performed on thick sections from the block prepared for electron microscopy, which were fixed in 3% glutaraldehyde and 2% paraformaldehyde. The tissue block for electron microscopy was dehydrated with ethanol and embedded in propylene oxide and epoxy resin to prepare ultra-thin sections, which were stained with uranyl acetate and lead and evaluated by electron microscopy.

For the kidneys, H&E staining was performed on samples from all of the patients using the method described above, and TB staining was conducted on samples from Patient 7. The skin, liver, lung, spleen, pancreas, and adrenal gland samples obtained from six of the seven patients were evaluated by H&E staining (except Patient 5).

**Results**

**Clinical characteristics**

The clinical characteristics of the seven patients are summarized in Table 1. The age at the time of death of the patients was 63—83 years. No blood relationships were observed between the patients. All of the patients suffered cardiac death. Patient 7 died of ventricular arrhythmias and the other six patients died of heart failure. The plasma α-gal A activity was 0.4—1.4 nmol/h/ml, which was 4—17% of the normal control activity [25]. Coro-

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Clinical characteristics of seven patients with cardiac Fabry disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient number</td>
<td>1</td>
</tr>
<tr>
<td>Age at death (year)</td>
<td>68</td>
</tr>
<tr>
<td>Cause of death</td>
<td>Heart failure</td>
</tr>
<tr>
<td>Plasma α-gal A activity (nmol/h/ml)</td>
<td>1.2</td>
</tr>
<tr>
<td>% of normal</td>
<td>14</td>
</tr>
<tr>
<td>Coronary angiography</td>
<td>Normal</td>
</tr>
<tr>
<td>Albuminuria</td>
<td>(−)</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.9</td>
</tr>
<tr>
<td>Cerebrovascular damage</td>
<td>(−)</td>
</tr>
<tr>
<td>Angiokeratoma</td>
<td>(−)</td>
</tr>
<tr>
<td>Acroparesthesias</td>
<td>(−)</td>
</tr>
<tr>
<td>Hypohidrosis</td>
<td>(−)</td>
</tr>
<tr>
<td>Corneal opacities</td>
<td>(−)</td>
</tr>
</tbody>
</table>

(−), Absence of the finding.
Terminal stage cardiac findings in patients with cardiac Fabry disease

Table 2

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Rhythm/HR (beats/min)</th>
<th>PR Interval (s)</th>
<th>QRS Duration (s)</th>
<th>Abnormal Q wave</th>
<th>SV1 + RV5 (mV)</th>
<th>Holter ECG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SR/90</td>
<td>0.24</td>
<td>0.20 (−)</td>
<td>2.2</td>
<td>Nonsustained VT</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>SR/72</td>
<td>0.26</td>
<td>0.18 (+)</td>
<td>4.1</td>
<td>Nonsustained VT</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>SR/83</td>
<td>0.22</td>
<td>0.20 (−)</td>
<td>2.6</td>
<td>Nonsustained VT</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Paced/62</td>
<td>Paced</td>
<td>Paced</td>
<td>Paced</td>
<td>Paced</td>
<td>Nonsustained VT</td>
</tr>
<tr>
<td>5</td>
<td>Paced/86</td>
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<td>Paced</td>
<td>Paced</td>
<td>Paced</td>
<td>Nonsustained VT</td>
</tr>
<tr>
<td>6</td>
<td>Paced/77</td>
<td>Paced</td>
<td>Paced</td>
<td>Paced</td>
<td>Paced</td>
<td>Nonsustained VT</td>
</tr>
<tr>
<td>7</td>
<td>SR/75</td>
<td>0.24</td>
<td>0.16 (−)</td>
<td>2.4</td>
<td>Nonsustained VT</td>
<td></td>
</tr>
</tbody>
</table>

HR, heart rate; SR, sinus rhythm; VT, ventricular tachycardia; (−), absence of the finding; (+), presence of the finding.

Electrocardiographic findings

The standard 12-lead ECG findings are shown in Table 2 and Fig. 1(A). Patient 4 had received a DDD pacemaker implantation because of sick sinus syndrome. Patients 5 and 6 had received DDD pacemaker implantations because of complete atrioventricular block. The remaining four patients showed normal sinus rhythm, but with first degree atrioventricular block (PR interval, 0.22–0.26 s). The four patients showed evidence of intraventricular conduction disturbances with QRS intervals of 0.16–0.20 s. Abnormal Q-waves were observed in Patient 2. Patient 2 also had left ventricular high voltage (SV1 + RV5 = 4.1 mV). Nonsustained ventricular tachycardia was observed on the Holter ECGs of all seven patients.

Echocardiographic findings

The echocardiographic findings are shown in Table 3 and Fig. 1(B, C). All patients showed evidence of...
LVH with an interventricular septal wall thickness of 14–20 mm and a left ventricular posterior wall thickness of 13–20 mm. However, localized thinning of the basal posterior wall of the left ventricle was observed in six patients (except Patient 7). The wall thickness of the thinned base of the left ventricular posterior wall was 4–7 mm. The left atrial dimension was increased to 45–52 mm in six patients (except Patient 7). The left ventricular end-diastolic dimension was also increased to 60–72 mm in six patients (except Patient 7).

The fractional shortening of the left ventricle decreased to 7–22% in all of the patients. Diffuse left ventricular hypokinesis was observed in all seven patients. In addition, Patients 1, 3–5 and 6 showed evidence of akinesis of the posterior wall, and Patient 2 showed evidence of dyskinesis of the posterior wall.

Figure 2  Macroscopic findings of the hearts from Patients 1 (A), 2 (B), 3 (C), 4 (D), 5 (E) and 6 (F). Hypertrophy of both ventricles, with particularly marked left ventricular hypertrophy, is present. These six patients who died of heart failure had localized thinning at the base of the left ventricular posterior wall (indicated by arrows).
Histologic studies of the heart

The heart weight in the seven patients was elevated to 575, 655, 760, 688, 510, 534 and 520 g, respectively. Hypertrophy of both ventricles was observed, along with marked LVH (Fig. 2). Diffuse deposition of translucent white material was observed grossly in the myocardium in all of the patients, and Patients 1–6 showed localized thinning of the base of left ventricular posterior wall (Fig. 2). All patients showed no evidence of organic stenosis of the coronary arteries.

Based on H&E staining, each patient had sarcoplasmic vacuolization and decidualation of myocardial cells, with fatty infiltration and diffuse fibrosis of the stroma in the left ventricle (Fig. 3A–F). Similar findings were observed in the right ventricle, left atrium, and right atrium, although the changes were milder than those in the left ventricle. The thinned region of the base of the left ventricular posterior wall observed in Patients 1–6 showed marked fibrosis with almost no myocardial cells observed (Fig. 3G–L). In addition, the remaining myocardial cells showed severe sarcoplasmic vacuolization.

Based on TB staining, accumulation of strongly osmiophilic substance was observed in the vacuolated regions identified by H&E staining, which was consistent with the staining pattern of glycosphingolipid that accumulates in Fabry disease (Fig. 4). In addition, the degree of accumulation varied based on location, with the left side of the heart showing a higher degree of involvement than the right side of the heart. Based on electron microscopy, all patients examined showed increased lysosomal inclusions with concentric lamellar configurations characteristic of Fabry disease affecting the cytoplasm of myocardial cells (Fig. 5).

The sarcoplasmic vacuolization observed with H&E staining was not seen in the coronary arteries or blood vessels between myocardial cells (Fig. 6A). Based on electron microscopy, no lysosomal inclusions with concentric lamellar configurations were observed in cardiac capillary endothelial cells (Fig. 6B).

Histologic findings of other organs

Sclerosis and atrophy of the kidneys was observed grossly in six patients (except Patient 7), which was thought to be due to peripheral circulatory failure caused by heart failure. Histologically, all patients showed no evidence of sarcoplasmic vacuolization in the endothelium or mesangial cells of the glomerulus and renal tubules. In kidney tissue from Patient 7, on which TB staining could be conducted, no strongly osmiophilic substance was present in the endothelium or mesangial cells of the glomerulus and renal tubules.

Figure 3  Light microscopic findings of the hearts from Patients 1 (A, G), 2 (B, H), 3 (C, I), 4 (D, J), 5 (E, K) and 6 (F, L). Based on hematoxylin and eosin staining (×100), all patients show sarcoplasmic vacuolization of the myocardial cells (A–F). The thinned region of the base of left ventricular posterior wall observed in these six patients shows marked fibrosis, and almost no myocardial cells are observed (G–L).
In the skin samples, none of the patients showed evidence of vacuolization in any component of the epidermis, secretory epithelium of the sweat gland in dermis, vascular endothelial cells, or fibroblasts. Concerning the liver, there was gross evidence of 'nutmeg liver'-like congestion and sclerosis in the patients who died of heart failure. Histologically, all patients showed no evidence of sarcoplasmic vacuolization in the hepatocytes, sinusoidal endothelial cells, or Kupffer cells. The lung wet weight was increased in all patients and there were macroscopic findings consistent with congestion and bleeding. Histologically, there was collapse and bleeding of alveolar cavities and intra-alveolar infiltration of hemosiderin phagocytes consistent with pulmonary congestion. However, there was no

**Figure 4** Light microscopic findings of the hearts from Patients 1 (A), 2 (B), 3 (C), and 4 (D). Based on toluidine blue staining ($\times400$), accumulation of strongly osmiophilic substance is observed in myocardial cells, which is consistent with glycosphingolipid accumulation.

**Figure 5** Electron microscopic findings from the heart of Patient 1. Panels (A) and (B) show typical lysosomal inclusions with a concentric lamellar configuration. The black bar indicates 3 $\mu$m in Panel (A), and 0.5 $\mu$m in Panel (B).
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Figure 6 Light and electron microscopic findings of blood vessels between myocardial cells from Patient 1. No sarcoplasmic vacuolization visualized by hematoxylin and eosin staining is observed in the vascular endothelial cells (A, indicated by arrow, ×200). Based on electron microscopy (B), no accumulation of lysosomal inclusions in the capillary endothelial cell is observed (indicated by arrow). The black bar indicates 3 μm (B).

sarcoplasmic vacuolization characteristic of typical Fabry disease in the lung. Although the spleen, pancreas, and adrenal glands were examined, no sarcoplasmic vacuolization was observed.

Discussion

In male patients with cardiac Fabry disease, abnormal ECG findings include sinus bradycardia, sick sinus syndrome, atrial fibrillation, shortened PR interval, atrioventricular block, intraventricular conduction disturbances, left ventricular high voltage, abnormal Q-waves, ST-T changes, and premature ventricular contractions [20–24]. In male patients with typical Fabry disease, Bass et al. [8] described many patients with LVH, and Colucci et al. [9] identified one patient with asymmetric septal hypertrophy and stenosis of the left ventricular outflow tract. In the seven patients with cardiac Fabry disease in this study, moderate to severe LVH was observed. In the setting of Fabry disease, glycosphingolipid accumulates in myocardial cells [1]. This accumulation is believed to be progressive, and may lead to LVH [8,13,15]. In this study, accumulation was observed in myocardial cells in patients with cardiac Fabry disease, and this accumulation may be the cause of LVH.

In six of the seven patients with cardiac Fabry disease in this study, localized thinning of the base of the left ventricular posterior wall was detected by echocardiography and confirmed at the time of autopsy. Histologically, the thinned region showed evidence of deciduation and marked fibrosis of the myocardium. Moon et al. [31] reported using gadolinium-enhanced cardiovascular magnetic resonance that myocardial fibrosis occurs in the base of the left ventricular infero-lateral wall in patients with Fabry disease. Yet pathological evidence of localized thinning of the base of the left ventricular posterior wall has not previously been reported in Fabry disease, including cardiac Fabry disease. Because no significant coronary artery stenoses were observed in any of the patients and no accumulation was observed in the coronary arteries or cardiac capillary endothelial cells, it is difficult to implicate myocardial ischemia as a cause of the thinning. Localized thinning of the base of left ventricular posterior wall has similar been reported in patients with Duchenne muscular dystrophy [32]. Cziner and Levin [32] hypothesized that the construction of myocardium differs in different regions of the left ventricular wall. Specifically, the run of myocardial fibers is net-like in the anterior wall, whereas in the base of the posterior wall the fibers are parallel. It is hypothesized that the base of the posterior wall becomes thin because of pressure and volume overload caused by differences in
the construction of the myocardial fibers. In the patients with cardiac Fabry disease in this study, it is possible that localized thinning occurred at the base of the left ventricular posterior wall through a similar mechanism. We reported that appearance of basal posterior left ventricular wall thinning is an important echocardiographic finding that precedes heart failure in patients with cardiac Fabry disease [33]. In contrast to the other six patients, the one patient with no thinning at the base of left ventricular posterior wall had no evidence of enlargement of the left atrium or left ventricle, and the left ventricular fractional shortening was the greatest of the seven patients. It suggests that localized thinning of the base of the left ventricular posterior wall may be related to the severity of left ventricular dysfunction.

With respect to left ventricular contractility in male patients with cardiac Fabry disease, Elleder et al. [20] described a patient with hypokinesis of the left ventricular posterior wall. Frustaci et al. [24] described a male patient with cardiac Fabry disease with hypokinesis of the midventricular and apical walls and dyskinesis of the midventricular inferior septum, with a decreased fractional shortening of 22%. In male patients with typical Fabry disease, Bass et al. [8] reported that 4 of 22 patients had decreased left ventricular contractility. All of the patients in this present study had diffuse asynnergy of the left ventricle with markedly reduced left ventricular fractional shortening.

Among male patients with cardiac Fabry disease, no glycosphingolipid accumulation in the coronary arteries or cardiac capillary endothelial cells has been observed in histologic examinations of the hearts of a total of three patients [20,21,23]. However, in typical Fabry disease, it has been reported that glycosphingolipid accumulation occurs in coronary arteries, endothelial cells, and smooth muscle cells of cardiac arterioles and capillary endothelial cells [2,4,5,7,9,18]. In the seven patients with cardiac Fabry disease in this study, no stenoses were identified by coronary angiography. In addition, there were no light microscopic findings of glycosphingolipid accumulation in the coronary arteries or blood vessels between myocardial cells, and no accumulation in cardiac capillary endothelial cells was observed by electron microscopy. In addition, there was no glycosphingolipid accumulation in the kidneys, skin, liver, lung, spleen, or adrenal gland. In the seven patients with cardiac Fabry disease in this study, accumulation of glycosphingolipid was observed in myocardial cells but not in the coronary arteries, cardiac arterioles, cardiac capillary endothelial cells or other organs examined. Therefore, we confirmed that cardiac Fabry disease is a unique pathologic entity.

Conclusions

The present study reports that severe left ventricular dysfunction with associated conduction disturbances and ventricular arrhythmias were observed in terminal stage patients with cardiac Fabry disease. Autopsies of the patients revealed the presence of LVH with localized thinning of the base of the left ventricular posterior wall. In contrast to typical Fabry disease, accumulation of glycosphingolipids occurs in myocardial cells but not in other organs.

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