Morphological Changes In Small Vessels On Endomyocardial Biopsy

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ABSTRACT

Small vessel disease has been described in various cardiac conditions including diabetes mellitus, amyloidosis, and connective tissue disease. Less well understood is the incidence and morphological features of small vessel disease in patients with myocardial disease of unknown etiology. This study examines the incidence, clinical presentation, and pathological changes of small vessel disease in patients with normal epicardial coronary arteries undergoing endomyocardial biopsy. Biopsy specimens in 110 consecutive patients were analyzed by light and electron microscopy. Small vessel abnormalities were present in 16 patients (14.6 percent) of whom five patients had associated hypertension and 11 patients had idiopathic small vessel disease. There were six males and 10 females with a mean age of 53 (26 to 76) years. Clinical presentations were arrhythmias, heart failure, or chest pain. The left ventricular ejection fraction was reduced (<50 percent) in 12 of these 16 patients. The morphological features of small vessel disease included marked thickening of the arterial wall owing to subendothelial deposits of heterogenous electron dense materials consisting of microfibrils, collagen and elastic fibers, cellular debris, and other amorphous substances. Subendothelial deposits comprised a mean 60 percent (40 to 76 percent) of the arterial wall thickness.

Introduction

Large epicardial coronary arteries are the most common site of pathological involvement, particularly from atherosclerosis. Small coronary arteries or arterioles also may become obstructed in patients with diabetes mellitus, amyloidosis, connective tissue diseases, and other pathological conditions which are less well understood. The incidence and clinical presentation of patients with small vessel disease are unknown. Obstruction of small vessels may impede coronary blood flow, and the clinical presentation would be indistinguishable from that of obstruction in large epicardial coronary arteries. To assess the pathological changes in small coronary vessels, endomyocardial biopsy specimens from 110 consecutive patients were studied by light and electron microscopy and analyzed by morphometric methods.
Materials and Methods

Clinical Evaluation

There were 110 consecutive patients included in this study, 64 males and 46 females with a mean age of 52 years and ranging from 14 to 76 years. The medical record of each patient was reviewed retrospectively. The most common indication for endomyocardial biopsy was suspected inflammatory myocarditis in patients with a recent onset (<one year) of unexplained heart failure which was usually associated with a reduced left ventricular ejection fraction. Other indications included chest pain, arrhythmias, sudden death, and assessment of doxorubicin cardiotoxicity and cardiac allograft rejection. All patients had normal epicardial coronary arteries by coronary arteriography.

Endomyocardial Biopsy

Cardiac catheterization was performed in all patients using standard techniques. At the conclusion of the procedure, multiple biopsy specimens were taken from the right ventricular septum. Three to six specimens of biopsy tissue were obtained in each patient. The specimens measured one to two mm in size and were immediately immersed in Bouin’s solution for light microscopy and in 2.5 percent buffered glutaraldehyde solution for electron microscopy. In select cases, direct immunofluorescence was also studied.

Pathologic Evaluation

All histological sections were stained with hemotoxylin and eosin, Masson trichrome, periodic acid-Schiff, and elastic van Gieson’s stains. In selected cases specimens were stained with Congo red and Prussian blue. Electron microscopic (EM) specimens were embedded in plastic resin. Thick sections of the EM specimens were stained with toluidine blue. Thin sections were taken from selected areas, stained with uranyl acetate and lead citrate, and viewed under a Hitachi electron microscope.

In addition to routine evaluation of endocardium, myocardium, interstitium, and intramural blood vessels, the diameter of myocardial cells and extent of interstitial fibrosis were measured morphometrically using Micro-plan II.* Specific scores were given to each biopsy. Myocardial hypertrophy was classified as “0” or absent (<15 μm), “1” or mild (15 to 20 μm), “2” or moderate (20 to 25 μm) and “3” or severe (>25 μm). Interstitial fibrosis was also scored as “0” or absent (<5 percent), “1” or mild (5 to 10 percent), “2” or moderate (10 to 20 percent) and “3” or severe (>20 percent). Myofibrillar degeneration was evaluated by electron microscopy. Although small vessel changes can be seen in light microscopic examination, the degree of involvement may only be evident on electron microscopic examination. The thickness of the subendothelial zone as compared to the total vessel wall thickness also was measured morphometrically.

Results

Pathological Classification of Endomyocardial Specimens

Cardiomyopathy: Biopsy specimens with myocardial hypertrophy greater than 15 μm, interstitial fibrosis of more than five percent, and myofibrillar degeneration on electron microscopy were considered to be consistent with a cardiomyopathic process.

Myocarditis: Active myocarditis was diagnosed on the basis of myocyte

* Laboratory Computer System, Inc. Cambridge, MA.
necrosis and inflammatory cell aggregates or lymphocytic infiltrates greater than five lymphocytes per high power field in 20 randomly selected areas. To minimize sampling error, a minimum of three samples were obtained for analysis from each patient.

*Amyloidosis:* Congo red stain of the biopsy specimens showed apple-green birefringent materials, and electron microscopy showed the presence of amyloid fibrils.

*Interstitial fibrosis:* Biopsy specimens showed only interstitial fibrosis without myocellular hypertrophy or conspicuous myofibrillar degeneration.

*Small vessel disease:* Intramural small arteries or arterioles (20 to 100 μm in diameter) showed marked wall thickening owing to widening of subendothelial zone and/or medial hypertrophy (figures 1 and 2). The subendothelial zone contained heterogenous electron dense materials, such as microfibrils, collagen and elastic fibers, cellular debris, or other amorphous substances (figures 3 and 4). Morphometric measurements of subendothelial deposits revealed that

**Figure 1.** Intramycocardial small arteriole showing widening of subendothelial zone and perivascular fibrosis. (×600)
PATHOLOGY OF SMALL CORONARY VESSELS

FIGURE 2. Intramyocardial small artery showing marked thickening of vessel wall with widening of subendothelial zone and medial hypertrophy. (×300)

FIGURE 3. Ultrastructure of arteriole showing marked widening of subendothelial zone (→ ←) composed of heterogeneous electron dense material. (×8,000)
these materials comprised a mean 60 percent (40 to 76 percent) of arterial wall thickness. Age related changes in intramyocardial arterioles has been demonstrated by Billingham et al. The normal thickness of the subendothelial zones varied with age, ranging from 12.5 percent in the 16 to 31 years age group, 29 percent in the 40 to 56 age years age group, and 37 percent in the 57 to 64 years age group. In patients with small vessel disease in our study, the thickness of the subendothelial zone was significantly greater than the results of age related changes reported by Billingham et al and showed no correlation with age.

Minimal or non-diagnostic changes: These biopsy specimens showed no evidence of myocellular hypertrophy or conspicuous interstitial fibrosis. Electron microscopic examination revealed only minimal myocardial changes.

The pathologic classification of endomyocardial biopsy specimens from these 110 consecutive patients examined in our institution was tabulated in Table I. There were 54 patients (49.1 percent) with pathological changes consistent with cardiomyopathy of whom 41 were idiopathic, five had doxorubicin cardiotoxicity, three had a history of alcohol abuse, two had heart disease which occurred in the peripartum period, one had a family history of cardiomyopathy, and two had connective tissue disease, one with scleroderma and one with systemic lupus erythematosus. Myocarditis was diagnosed in 12 patients (10.9 percent). Amyloidosis was confirmed in two patients (1.8 percent). Ten patients (9.1
Table I

Incidence of Pathological Diagnosis in 110 Consecutive Patients Undergoing Endomyocardial Biopsy

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of Patients</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiomyopathy</td>
<td>54</td>
<td>49.1</td>
</tr>
<tr>
<td>Idiopathic</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Adriamycin</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Peripartum</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Familial</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Scleroderma</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Lupus erythematosus</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Myocarditis</td>
<td>12</td>
<td>10.9</td>
</tr>
<tr>
<td>Amyloidosis</td>
<td>2</td>
<td>1.8</td>
</tr>
<tr>
<td>Small vessel disease</td>
<td>16</td>
<td>14.6</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Normotensive</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Interstitial fibrosis</td>
<td>10</td>
<td>9.1</td>
</tr>
<tr>
<td>Minimal or non-diagnostic</td>
<td>16</td>
<td>14.6</td>
</tr>
</tbody>
</table>

percent) had only interstitial fibrosis. Sixteen patients (14.6 percent) had significant intramural small vessel abnormalities. Five of these patients had a history of hypertension but 11 patients had no known cause and were considered to be idiopathic. Sixteen patients (14.6 percent) showed minimal or non-diagnostic changes.

Morphometric Analysis of Endomyocardial Specimens

Morphometric measurements for each pathologic diagnosis are shown in table II. Specimens consistent with the diagnosis of cardiomyopathy had the greatest degree of myocellular hypertrophy. The small vessel disease group had less myocellular hypertrophy but slightly more interstitial fibrosis than those with a pathologic diagnosis of cardiomyopathy. In the 16 patients with small vessel disease, there were no qualitative or quantitative differences in the morphological changes between patients with and without hypertension. The morphological changes in the small vessels included marked thickening of the arterial wall owing to subendothelial deposits of heterogenous electron dense materials consisting of microfibrils, collagen and elastic fibers, cellular debris, and amorphous granular substances. Subendothelial deposits comprised a mean 60 percent (40 to 76 percent) of arterial wall thickness. Nine of these patients had additional myocardial changes which were similar to those seen in cardiomyopathy. Four other patients showed interstitial fibrosis only and the remaining three patients had no conspicuous myocardial changes.

Clinical Pathological Correlation of Small Vessel Disease

The clinical presentation of patients with small vessel disease was varied.

Table II

Morphometric Measurements of Various Pathological Diagnosis on Endomyocardial Biopsy

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Myocellular Hypertrophy (μm)</th>
<th>Interstitial Fibrosis Scores</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiomyopathy</td>
<td>18.5 ± 2.7</td>
<td>11.3 ± 3.8</td>
<td>1.50</td>
</tr>
<tr>
<td>Myocarditis</td>
<td>15.2 ± 2.6</td>
<td>9.6 ± 3.6</td>
<td>1.29</td>
</tr>
<tr>
<td>Small vessel disease</td>
<td>15.9 ± 3.4</td>
<td>13.8 ± 3.2</td>
<td>1.63</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>16.5 ± 3.7</td>
<td>13.5 ± 3.2</td>
<td>1.80</td>
</tr>
<tr>
<td>Normotensive</td>
<td>15.5 ± 3.2</td>
<td>14.0 ± 3.9</td>
<td>1.85</td>
</tr>
<tr>
<td>Interstitial fibrosis</td>
<td>12.4 ± 1.4</td>
<td>14.1 ± 4.9</td>
<td>1.86</td>
</tr>
<tr>
<td>Minimal of non-diagnostic</td>
<td>13.0 ± 1.8</td>
<td>3.8 ± 3.6</td>
<td>0.53</td>
</tr>
</tbody>
</table>

*Mean ± standard deviation
There were six males and 10 females with a mean age of 53 years. Small vessel disease of the myocardium occurred more frequently in female patients, although overall more males underwent endomyocardial biopsy. There was no significant age difference between patients with small vessel disease and others undergoing endomyocardial biopsy. Overall, eight patients presented with arrhythmias, including two patients with associated heart failure and one patient with associated chest pain. Five other patients presented with the recent onset of heart failure and three patients with chest pain. Thus, arrhythmias were the most frequent clinical presentation in patients with small vessel disease. Hemodynamic studies revealed that 12 of these 16 patients with small vessel disease had a reduced left ventricular ejection fraction less than 50 percent.

Discussion

Although the most common indication for endomyocardial biopsy in this study was clinically suspected myocarditis, the actual incidence of biopsy proven myocarditis was only 10.9 percent. The majority of our patients were diagnosed as having non-specific cardiomyopathy based on the presence of myocellular hypertrophy, interstitial fibrosis, and myofibrillar degeneration. The cause of cardiomyopathy in the majority of these patients was unknown. In other patients, cardiomyopathy was associated with certain clinical states, such as doxorubicin treatment, alcoholism, pregnancy, scleroderma, or systemic lupus erythematosi.

Surprisingly, a significant number of patients were found to have small intramural coronary vessel abnormalities. In the present series of 110 consecutive patients, 16 patients (14.6 percent have been identified) with abnormal morphological changes in their small coronary vessels. This incidence of small vessel disease may be underestimated because arterioles were not found in all biopsy specimens. Some 30 percent of these patients with small vessel disease had a history of hypertension while 70 percent had no known associated conditions. The presence and significance of small vessel disease in coronary vessels is rather controversial. Relatively few reports relating the incidence and the clinical presentation of patients with small vessel disease of the myocardium are present in the literature. Weiss and Fenoglio reported 12 patients with histologic evidence of small vessel disease in a group of 100 patients. McReynolds and Roberts described abnormalities of intramural coronary arteries in 75 percent of patients with hypertrophic cardiomyopathy. Richardson et al demonstrated the presence of abnormal intramural small arteries and arterioles in patients who had undergone endomyocardial biopsy. Some of the controversy concerning the incidence of small vessel disease may be related to evaluation of light microscopic specimens only, since the degree of involvement in small vessels may be appreciated better by electron microscopic examination.

Small arterial changes are frequently seen in the kidney in patients with hypertension, but the role of hypertension in the production of intramural small coronary vessel changes is controversial. Donomae et al have reported in patients with hypertension that intramyocardial arteries were rarely involved with the type of lesions that were seen in small vessels of the kidney. In contrast, Blumenthal et al described a “hemodynamic lesion” in small coronary arteries in patients with hypertension. This lesion consisted of a fibrous or fibroblastic intimal thickening with a variable PAS reaction and in arterioles as hyaline thickening with PAS positive fibrils. These “hemodynamic lesions” were
thought to be associated with hypertension. In our present study, five of 16 patients with small vessel disease had a history of hypertension, suggesting that a hemodynamic factor may contribute to the pathogenesis of small coronary vessel changes. On the other hand, 11 of 16 patients had no significant associated conditions and, thus, the etiology of this idiopathic small vessel disease was unknown.

Other etiologies have been suggested as a cause of small vessel disease. There may be a causative relationship between an episode of myocarditis and subsequent development of small vessel disease. Coxsackie B viral infection has been shown to produce extensive arteritis and capillary damage in experimental animals. James also has suggested that small coronary vessel disease may be causative for some obscure cardiomyopathies. Hamby et al found lesions similar to those described by Blumenthal et al but in patients with cardiomyopathy who were not hypertensive. Small coronary arteriolar changes may represent artheriolar cardiosclerosis, part of a generalized process of arteriosclerosis. Factor and Sonnenblick have suggested that spasm of small coronary vessels may lead to myocardial damage and cardiomyopathy.

Secondary small vessel disease has been described in association with diabetes mellitus, amyloidosis, and connective tissue diseases. Small vessel disease was shown to be 2.5 times more frequent in diabetics than in non diabetic patients. The basement membrane of small vessels in diabetic patients was frequently found to be thickened up to 1000 angstroms. None of our patients with small vessel disease had a history of diabetes mellitus. Moreover, no significant thickening of basement membranes of small coronary vessels was observed in these patients. Two patients in our present series with amyloidosis had amyo-
cardiomyopathy may represent a heterogeneous group of diseases with similar myocardial abnormalities. Based on this study, small vessel disease may be one of many causes of cardiomyopathic process, since more than half of these biopsy specimens showing small vessel abnormalities were associated with myocardial changes similar to those seen in cardiomyopathy, while others had interstitial fibrosis or minimal myocardial changes. These myocardial changes may represent various stages of involvement from small vessel disease. This group of patients with small vessel disease can be separated from those with cardiomyopathy of unknown cause. It has been suggested that the association of small vessel disease and cardiomyopathy may carry a poor prognosis, however, further studies are necessary to substantiate this observation.

Conclusion

1. The incidence of small coronary vessel disease in 110 consecutive patients undergoing endomyocardial biopsy was 14.6 percent. 
2. Arrhythmias were the most frequent clinical presentation followed by recent onset of heart failure and chest pain. 
3. Ultrastructurally, the subendothelial zone was markedly thickened and composed of heterogenous electron dense materials which may result in loss of vessel wall compliance and luminal narrowing. 
4. The consequence of these changes may lead to myocardial damage.

References