Circulating $\alpha$-actin in non-insulin-dependent diabetics with autonomic dysfunction

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Abstract

Silent myocardial ischemia in non-insulin-dependent diabetic patients occurs frequently in association with autonomic dysfunction, suggesting that diabetic neuropathy may be involved in the development of this disorder. Repeated episodes of silent myocardial ischemia can induce myocardial necrosis. Recently, actin was detected with Western blotting in the serum of patients with acute myocardial infarction and angina pectoris. We found that a large proportion of non-insulin-dependent diabetic patients with neuropathy also have detectable circulating concentrations of $\alpha$-actin, and therefore suggest that the determination by immunoblotting of serum $\alpha$-actin in such patients is an effective method to detect myocardial cell suffering and to identify patients that may need special consideration.

Keywords: Diabetic neuropathy; Silent myocardial ischemia; $\alpha$-actin

1. Introduction

Ischemic heart diseases such as acute myocardial infarction and angina pectoris are more frequent in non-insulin-dependent diabetic patients that in nondiabetics [1]. Moreover, many studies have shown that the prevalence of silent myocardial ischemia during daily activities in asymptomatic diabetic patients is very high [2]. Silent myocardial ischemia occurs frequently in association with autonomic dysfunction, suggesting that diabetic neuropathy, which appears to be caused by modifications in nerve cytoskeletal proteins [3], may be involved in the mechanism of this disorder [4]. In diabetic patients with cardiac autonomic neuropathy, repeated episodes of silent myocardial ischemia can induce myocardial necrosis and sudden death [5].

Immunoblotting was recently used to detect $\alpha$-actin in the serum of patients with cardiac ischemia [6,7]. This highly sensitive technique involves the electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets, and the
subsequent demonstration of antibody binding. Immunoblotting can detect amounts of protein as low as 0.1 to 1 ng, and is becoming commonplace in serological diagnoses based on the detection of immunocomplexes. We used Western blot assays to detect the presence of cardiac isoform α-actin as a marker of myocardial injury in the serum of non-insulin-dependent diabetic patients with autonomic neuropathy.

2. Materials and methods

2.1. Control group and patients

Control assays were done in 30 serum samples (12 (40%) men, 18 (60%), women, mean age 51 ± 8 years) obtained from the Granada Provincial Blood Bank. Control samples were from non-diabetic patients. Serum α-actin was also analyzed in 53 patients diagnosed with non-insulin-dependent diabetes by the Endocrinology Service of the University of Granada Hospital. Diabetic patients with a history of angina pectoris or myocardial infarction were excluded. The patients were classified into two groups according to the results of autonomic dysfunction tests. Table 1 shows the characteristics of these groups. Group I consisted of 24 non-insulin-dependent diabetic patients with autonomic dysfunction, and Group II consisted of 22 non-insulin-dependent diabetic patients with no such dysfunction. Autonomic function was assessed by heart rate response to the Valsalva maneuver, deep breathing, and upright posture, as well as by systolic blood pressure response to upright posture [2]. An abnormal response on two or more of these tests was considered positive evidence of autonomic dysfunction. Blood samples from all diabetic patients and controls were centrifuged at 500 × g for 5 min to obtain approximately 1 ml of serum, to which was added 15 μl thimerosal (0.02%) to prevent contamination. The serum samples were frozen at −20°C in aliquots of 500 μl until analysis. As a control, we used total human cardiac proteins obtained from cardiac biopsies with a modified method of Lewis et al. [8].

2.2. Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Serum samples from patients and controls, and total cardiac muscle proteins, were resuspended in Laemmli’s sample buffer [9] and analyzed in duplicate by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) in a Mini Protean II cell (BioRad, Irvine, CA, USA) at 60 mA for 1 h at room temperature. One gel from each sample was used for densitometric analysis, and the other for immunoblot analysis. The gels for densitometric analysis were stained with Coomassie Blue R-250 and destained according to Laemmli et al. [6] (all reagents were obtained from Biorad). The gels were dried on filter paper (Whatman, Maidstone, England) with a Biorad 583 drier.

Table 1
Characteristics of diabetic patients and control group

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>30</td>
<td>24</td>
<td>22</td>
</tr>
<tr>
<td>Sex F/M</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>18 (60%)</td>
<td>13 (54.1%)</td>
<td>10 (45.46%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>12 (40%)</td>
<td>11 (45.9%)</td>
<td>12 (54.54%)</td>
</tr>
<tr>
<td>Serum α-actin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>1 (3.33%)</td>
<td>18 (75%)</td>
<td>3 (13.63%)</td>
</tr>
<tr>
<td>Negative</td>
<td>29 (96.66%)</td>
<td>6 (25%)</td>
<td>19 (86.36%)</td>
</tr>
<tr>
<td>Retinopathy</td>
<td></td>
<td>5 (20.83%)</td>
<td>3 (13.63%)</td>
</tr>
<tr>
<td>Nephropathy</td>
<td></td>
<td>2 (8.33%)</td>
<td>1 (4.54%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td>9 (37.50%)</td>
<td>6 (27.27%)</td>
</tr>
</tbody>
</table>

Control group, non-diabetic patients; Group I, NIDDM patients with autonomic dysfunction; Group II, NIDDM patients without autonomic dysfunction.
2.3. Immunoblotting

The gels for immunoblot analysis were separated electrophoretically and transferred to nitrocellulose paper by applying a current of 30 V at room temperature for 12 h in 25 mM Tris-HCl (pH 8.3), 192 mM glycine, and 20% (vol/vol) methanol. The blots were treated with a blocking solution (20 mM Tris, 0.9% NaCl, 10% non-fat milk), washed with 10 mM Tris-buffered saline (pH 7.4) in 0.05% Tween 20, and then incubated with a 1:200 dilution of anti-sarcomeric actin monoclonal antibody (GR-ICOR-2) [10] for 3 h at room temperature. Positivity was detected with hors eradish peroxidase (HRP)-conjugated goat antibody to mouse immunoglobulin G (IgG) (Sigma), and developed with 4-chloronaphthol as described in the protocol of the immunoblot assay kit (BioRad Immunoblot AGR-HRP Assay Kit). The protein bands revealed by immunoblotting were quantified densitometrically using a standard curve [7]. Fisher’s exact test (4F BMDP) was used to compare the α-actin variable between controls and diabetic patients, and between diabetic patients with and without autonomic dysfunction. The Chi-squared test (4F BMDP) was used to analyze significance of the differences in age and sex between diabetic patients with and without autonomic dysfunction.

3. Results

We found that of 24 diabetic patients with a positive neuropathy test, 18 (75%) were positive for serum sarcomeric actin. The mean concentration of sarcomeric actin in the positive group was 20 mg/l (8.54% ± 4.1% of the densitometric value), with a range of 10.6 mg/l to 31.3 mg/l. In contrast, only 3 (13.6%) of the 22 diabetic patients with a negative neuropathy test had detectable serum concentrations of sarcomeric actin. The mean concentration of sarcomeric actin in these 3 patients was 15.3 ± 6.8 mg/l (6.2%), with a range of 5.6 to 18.7 mg/l. Of the 30 control samples from non-diabetic patients, 29 were negative for sarcomeric actin, and only one produced a single faint band (1.8%) of the 43 kDa level in the immunoblotting assay. Statistical analyses showed no significant differences in age and sex between diabetic patients with and without autonomic dysfunction.

However, significant differences were found in sarcomeric actin between controls and diabetic patients (P < 0.0001), and between diabetic patients with and without autonomic dysfunction (P < 0.0001).

4. Discussion

Experimental and clinical studies have shown that diabetes may result in alterations in cardiac protein composition [11]. Decreases in myosin heavy and light chain, troponin I, and actin were found in diabetic rats with myocardial dysfunction [12]; these proteins can be detected in the serum of patients with myocardial damage [7,13–16]. Increases in circulating cardiac myosin light chain [15] and tropomyosin [14] have been found with radioimmunoassay in patients with acute myocardial infarction. Enzyme-linked immunoassay has also been used to detect cardiac troponin T after acute myocardial infarction [16], and to detect...
troponin I in perioperative myocardial infarction [13]. Recently, Western blotting was used to detect actin, a 43 kDa molecule representing the major component of cardiac thin filament, in the serum of patients with acute myocardial infarction [6] and angina pectoris [7]. Our results in non-insulin-dependent diabetic patients suggest that the presence of circulating sarcomeric actin may reflect a process of myocardial cell damage. However, the low circulating levels of sarcomeric actin in diabetes patients contrasts with the high levels found in prolonged angina [7] and anterior acute myocardial infarction [6], suggesting that the degree of myocardial injury in diabetic patients is lower.

The Western blot assay developed in this study is an informative source of data on myocardial cell damage in diabetic patients with autonomic neuropathy. Because the circulating levels of sarcomeric actin we found in these patients may be related with silent myocardial ischemia, the finding of sarcomeric actin in the serum of non-insulin-dependent diabetic patients may indicate a high risk of acute myocardial infarction and/or angina pectoris, and could be used to identify diabetic patients that may need special consideration.

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References


