Morphologic Findings of the Ischemic Myocardium

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Abstract

This study assesses the relation between qualitative and quantitative findings of myocytes and interstitial connective tissue in the ischemic heart disease. Qualitative and quantitative changes of myocytes and interstitial connective tissue were studied on the serial cross myocardial sections from 20 autopsied hearts with ischemic lesions, stained by immunohistochemistry using a monoclonal antibody (von Willebrand factor) and with hematoxylin-eosin method. Myocardial sections included proximal and distal part of occlusion and area of occlusion of coronary vessels. The volume densities (V_V) of the cardiac myocytes and interstitial fibrosis in the group with coronary occlusion were examined stereologically and compared with control group. The findings showed a significant reduction in the volume density of myocytes and an increase in the volume density of interstitial fibrosis in patients with coronary occlusion compared with control group. Significant reduction in the volume density of myocytes and an increase in volume density of interstitial fibrosis were greater in the distal part of occlusion and area of occlusion, than in the proximal part of the occlusion. Our stereological results give useful quantitative information's of changes in myocardium parts during coronary occlusion as well as in normal conditions, and represent objective proof of significant changes in ischemic myocardium described by qualitative analyses.

KEY WORDS: anemia, diabetes mellitus, heart failure, kidney failure

Introduction

Ischemic heart disease is a complex and diverse clinical syndrome in which an imbalance between blood supply and demand is created by complete or partial occlusion of a major epicardial coronary artery, resulting in myocardial infarction or multiple isolated sites of tissue injury. In addition, alteration of the intramural arterial

branches of the coronary vasculature or defects of the microcirculation may generate varying degrees of ischemia, with reflect the characteristics of myocytes loss in myocardium. On this basis, interstitial fibrosis have recently been as quantitative definitions of those aspects of myocyte loss (1,2,3). Interstitial fibrosis reflects widening on the extracellular space with collagen deposition between groups of myocytes, as a consequence of diffuse myocyte cell death. Myocardial autopsy techniques have extended our knowledge of the relation between morphologic findings and clinical aspects in various types of heart disease. The myocardium is composed of cardiac myocytes, interstitium, and capillaries which are enmeshed in a complex and extensive array of connective tissue structures. Morphometric studies in animal models and in human hearts have suggested that inadequate growth of the coronary microvascular bed is one factor limiting myocardial perfusion with consecutive reduction of myocytes and an increase of interstitial connective tissue (4,5,6,7,8). The present work aims to study stereologically changes in the human myocardium with occlusion of coronary arteries described by qualitative analysis. Morphologic changes of the myocardium myocytes and interstitially connective tissue, as a constituents of myocardium, in a various pathological conditions have been studied by many authors (9,10,11).

Material and Methods

Twenty human autopsied hearts based on macroscopic verification changes of coronary arteries devided in two groups: with coronary occlusion and control group (without occlusion or stenosis). For microscopic analysis cross sections of myocardium were fixed in 10% formaldehyde, embedded in paraffin, serially sectioned at 5-6 μm of thickness and stained with antibodies directed against von Willebrands factor (immunohistochemical method) and hematoxylin-eosin method (HE). Histological analysis was obtained using microscope Nikon with installation of digital camera. Stereogical analysis was obtained in serially myocardial sections with coronary blood vessels stained with immunohistochemical method (in the proximal and distal part of occlusion and area of occlusion) using test system M 42 (11). Number of samples was calculated according to De Hoff. By means of standard stereological procedures were calculated volume density of myocytes ($V_{V\,mv}$) and volume density of interstitial connective tissue ($V_{V\,int}$). Values are presented as the mean ±SEM. Statistical differences between the examined groups were determined by Student's t-test. Differences were considered significant for $p < 0,05$. The investigations were done with respect to ethical standards regulated by Helsinki Declaration.

Results

The results shows the qualitative changes in the myocardium with coronary occlusion:
hypertrophy, atrophy and myocytes necrosis with proliferation of interstitially connective tissue (Figure 1). Although some fibrosis was found in the control hearts (Figure 2), the density of fibrosis was greater in patients with coronary occlusion. Table 1 presents the values of volume density (VV) for cardiomyocytes (VV mv) and volume density of interstitial fibrosis (VV int) in area of occlusion (a), below of occlusion (b) and above of oc-

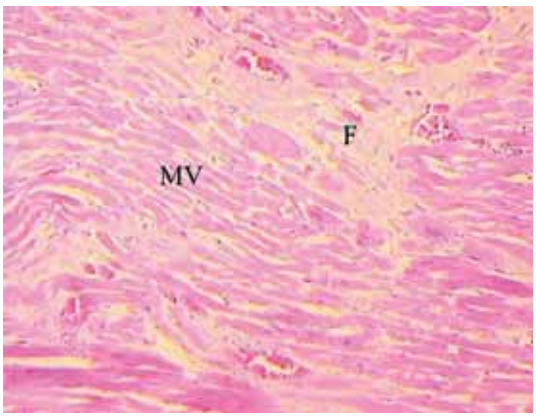

FIGURE 1. Area below of occlusion of the left coronary artery. Lesions of myocytes (MV) and interstitial fibrosis (F). HE, 100X

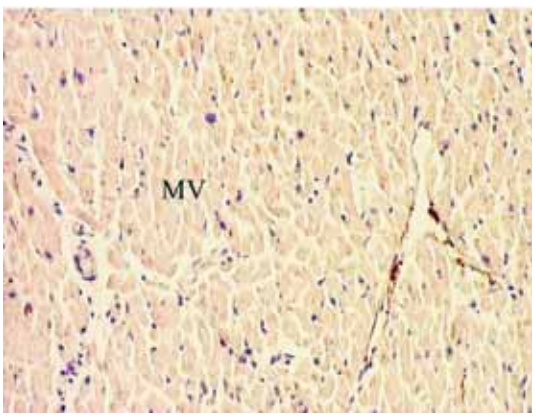

FIGURE 2. Myocardium in the control group with normal myocytes (MV) and minimal fibrosis. Immunohistochemistry using a monoclonal antibody (von Willebrand factor). 100X

MYOCARDIUM								
CONTROL GROUP (K)			CORONARY OCCLUSION (O)					
K	O		K	O		K	O	
$\bar{x}$ ±SEM	$\bar{x}$ ± SEM (a)	t p	$\bar{x}$ ±SEM	$\bar{x}$ ±SEM (b)	t p	$\bar{x}$ ±SEM	$\bar{x}$ ±SEM (c)	t p
$V_{V\ mv}$ 0,673 ±0,024	$V_{V\ mv}$ (a) 0,472 ±0,054	6,256 p<0,0001*	$V_{V\ mv}$ 0,673±0,024	$V_{V\ mv}$ (b) 0,454±0,021	6,160 p<0,0001*	$V_{V\ mv}$ 0,673±0,024	$V_{V\ mv}$ (c) 0,538 ±0,021	4,345 p<0,0001*
$V_{V\ int}$ 0,187 ± 0,011	$V_{V\ int}$ (a) 0,342 ±0,048	5,346 p<0,0001*	$V_{V\ int}$ 0,187 ±0,011	$V_{V\ int}$ (b) 0,346±0,017	5,858 p<0,0001*	$V_{V\ int}$ 0,187 ±0,011	$V_{V\ int}$ (c) 0,307 ±0,023	4,030 p<0,0001*

$V_{V\ mv}$-volume density of myocytes
$V_{V\ int}$-volume density of interstitial fibrosis
$\bar{x}$ -arithmetic mean
SEM-standard error of mean
*statistical significant differences

a-area of occlusion
b- below of occlusion
c- above of occlusion

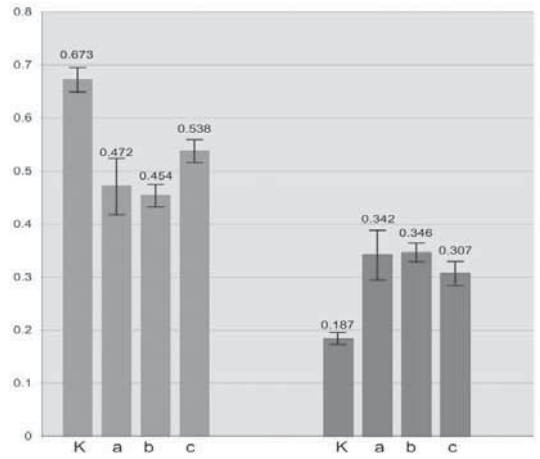

FIGURE 3. Volume density of myocytes (light gray) in the control group (K) and the group with coronary occlusion: in area of occlusion (a), below of occlusion (b) and above of occlusion (c). Volume density of interstitial fibrosis (dark gray) in the control group (K) and group with coronary occlusion in area of occlusion (a), below of occlusion (b) and above of occlusion (c).

clusion (c) for the group with coronary occlusion and control group (K), and statistical valorization of data. Volume density of myocytes in area of occlusion (a) was 0,472±0,054, below of occlusion (b) was 0,454±0,021, and above of occlusion (c) was 0,538±0,021. We established statistical significant differences for all levels comparing to control group (p<0,0001). Volume density of interstitial tissue in area of occlusion (a) was 0,342±0,048, below of occlusion (b) was 0,346±0,017, and above of occlusion (c) was 0,307±0,023. Comparing to control group all differences were significant (p<0,0001). The quantitative results showed a significant reduction in the volume density of myocytes and an increase in volume density of interstitial fibrosis in patients with occlusion compared with control group. Reduction in the volume density of myocytes and an increase in volume density of interstitial fibrosis was greatter in the distal part of occlusion and area of occlusion, than in the proximal part of occlusion. Figure 3. presents the same values of volume density for cardiomyocytes and volume density of interstitial fibrosis for the group with coronary occlusion and control group, and statistical valorization of data.

Discussion

The changes of the human myocardium with alteration of coronary circulation have always attracted the attention of morphologists as well as experts of many clinical disciplines. In the last years the number researches into the determination of quantitative changes in the myocardium, particularly the cardiac myocytes, and interstitial connective tissue, in the state of coronary stenosis or occlusion has considerately increased (1,2,3,4). Changes in the ischemic myocardium are in correlation with grade of stenosis or occlusion of coronary arteries. The numerous studies described the relationship between quantitative morphologic findings in myocardium and left ventricular contractile function in patients with idiopathic dilated cardiomyophaty (5,6,7,8,9). The present study describes, validates, and applies a histological

method and a morphometric technique to the measurement of changes of myocardial parts in a state with compromitation of coronary circulation. In the present study morphologic changes of the ischemic myocardium were quantitated by light microscopy. Quantitative results of our research showed a significant reduction in the volume density of myocytes and significant an increase in the volume density of interstitial connective tissue in patients with occlusion compared with control group. It's mean, that inadequate myocardial perfusion in the group with coronary occlusion result with consecutive reduction or necrosis of myocytes and proliferation of interstitial connective tissue (10,11). Although some fibrosis was found in the control hearts, the density of fibrosis was greater in patients with coronary occlusion. Area of occlusion and area below of occlusion are more affected by ischemia than area above of occlusion. For this reason, a reduction in the volume density of myocytes and an increase in volume density of interstitial connective tissue were greater in the distal part of occlusion and area of occlusion, than in the proximal part of the occlusion. Our stereological results correlate with results of qualitative analysis. With regard that the volume density is quantitative indicator of volume size in unit of organ volume, we consider that our stereological examines, objectively, give the proof of significant changes described by qualitative morphologic analysis.

Conclusion

Changes observed in myocardium, under ischemic conditions point, undoubtedly, to the fact of their active participation in the ischemic processes. Our quantitative study can be basis to plan of prospective clinical-morphological studies, based on comparison of the same changes in the ischemic myocardium obtained with morphometric analysis and by clinical methods. It can be useful for explanation of morphofunctional states of the myocardium in relation with arterial hypertension, pulmonary hypertension, and others form of heart hypertrophy.

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