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FIBROSIS PATTERNS IN DILATED CARDIOMYOPATHY AND ITS CLINICAL CORRESPONDENT

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Abstract

Introduction: Dilated cardiomyopathy (DCM) is a primary myocardial disease defined by the presence of heart dilation associated with left ventricular systolic dysfunction. Myocardial fibrosis is an important factor in the development and progression DCM. Extracellular matrix (ECM) is playing a central role in this condition. Aim: to describe the changes in the interstitial space of myocardial specimens in patients with DCM. Methods: We performed a retrospective study were we included all autopsied patients in our department with diagnosis of DCM (125 patients) during a period of 2 years. Results and Discussions: The main comorbidities encountered in DCM patients are pulmonary edema, atherosclerosis, stroke and hepatic impairment. The pattern of fibrosis is diffuse or focal, with a predominant perivascular topography. Cardiac magnetic resonance (CMR) is currently the method of choice for cardiac fibrosis assessment in alive patients. Conclusion: DCM requires a strong financial support because of decompensated heart failure (HF) and are a frequent indication for heart transplantation.

Key words: extracellular matrix, interstitial fibrosis, topography, comorbidities, cardiac magnetic resonance.

INTRODUCTION

Dilated cardiomyopathy (DCM) is a primary myocardial disease defined by the presence of heart dilation associated with left ventricular systolic dysfunction. [Mann DL, et al., 2015] [Juneja R, PM. Nambiar,2017]

Although a universal definition of cardiomyopathy is currently not accepted, this condition is a major cause of heart failure (HF) and a major cause of morbidity and mortality. [Suvarna SK., 2013]

The prevalence of dilated cardiomyopathy in the general population is unknown, but clearly it varies by age and geographical area.

The onset of the disease occurs most frequently in adulthood (20-60 years) with a mean age at presentation at about 50 years. [Fuster V, 1994], [J.C.E. Underwood, 2007]

Clinical presentation can be without symptoms, but pathophysiological, DCM causes global systolic impairment in the absence

of abnormal ventricular loading conditions (hypertension, valvular disease or coronary vascular disease. Right ventricular dilation and dysfunction may be present, but are not required for the diagnosis. DCM is characterized functional by systolic dysfunction, leading to a diminished stroke volumes, elevated left ventricular (LV) end-systolic and end-diastolic volumes, diminished ejection fraction, increased ventricular chamber dimensions and wall tension, and thinning of LV wall. (Mills S.E., 2010]

Myocardial fibrosis is an important factor in the development and progression of pathological changes of DCM, extracellular matrix (ECM) playing a central role. [Mihailovici AR, et al., 2017]

Several studies have shown that treatment with angiotensin converting enzyme inhibitors, angiotensin II receptor blockers or mineralocorticoid receptors antagonists can cause regression of the collagen in the ECM and other specific therapies that target MMP/TIMP system could have beneficial in the future. [Louzao-Martinez L, et al., 2016]

From primary DCM more than half of them have a familial background (especially when it is observed in more than 2 family members). [Apetrei E., 2015]

Also familial diseases (with autosomal dominant transmission) should be suspected when there is a history of premature death by cardiomyopathies or malignant arrhythmia or myopathies.

Recent studies have noted that dilated cardiomyopathy is one of the most common forms of myocardial disease, largely irreversible, with an estimated prevalence of 1:2500; it is the third most common cause of heart failure and the most common cause of heart transplant. According to some authors, idiopathic dilated cardiomyopathy is the most common cause of congestive heart failure in young people, with an estimated prevalence in the USA of at least 36.5 to 100 000.

Secondary DCM may occur in the context of other cardiovascular diseases (coronary heart disease, tachyarrhythmia, myocarditis), infectious diseases (viral, bacterial, fungal or parasitic), endocrine disorders (hyperthyroidism, pheochromocytoma, Cushing syndrome, growth hormone excess), diabetes mellitus, inflammatory conditions, autoimmune diseases, lysosomal storage disease, during the usage of toxic substances (alcohol, cocaine, amphetamines, cobalt, lead, lithium, mercury, beryllium metisergide), in hypothermia, irradiation, malnutrition, chemotherapy, thermic shock, amyloidosis and sleep apnea. [Apetrei E., 2015]

Knowledge of structural changes in myocardial cells and the myocardium as a whole could explain the pathophysiology of the disease and allow a correct therapeutic approach.

AIM

In this paper, we tried to describe the changes in the interstitial space of myocardial specimens in patients with DCM.

MATERIALS AND METHODS

Our study was conducted in the Pathology Department from County Hospital of Oradea with the consent of our head of department and the ethical committee. We included a total of 125 patients who were autopsied between 2015 – 2016 and diagnosed with DCM from a total of 263 dead patients that had diagnosis of DCM. The exclusion criteria were trauma death and lack of DCM in diagnosis in final autopsy report.

The autopsies were performed according to the proper legislation and with the consent of caregivers.

Myocardial tissue fragments were harvested from both the left ventricle (LV) and from the right ventricle (RV) for the histopathological histochemical. For the control group, we gather 15 cardiac fragments from corpses without severe cardiac pathology.

Myocardial fragments were kept in 10% buffered formalin for proper fixation (for minimum 24 hours), oriented and trimmed. Afterwards, the samples were processed for approximately 12 hours in an automatic tissue processor (Leica TP1020) and embedded in paraffin. Using a Leica manual microtome and Microm HM325 (equipped with a section transfer system), 5 µm thick sections were displayed on slides and left a half day for drying and de-waxing. The slides were manually stained using basic Hematoxylin – Eosin (HE), and special stains for collagen fibers (Masson's trichrome, trichrome Mallory and Van Gieson). A coverslip was mounted on every slide using BioMount reagent. For examination we used Leica DM 1000 optical microscope and the Nikon E600 polarized light microscope. The photos were taken using the camera attached to the microscope Leica DM 1000 microscope and using the camera software for clearing the images.

RESULTS

In this study 125 dead patients were autopsied and met the inclusion criteria. The majority of these patients were admitted to Internal Medicine Clinic of the same hospital as seen in Tabel 1. Of course, the majority of DCM patients were admitted in Cardiology, but only a small percentage of those were autopsied (more than 3 quarters were exempt from autopsy because of known and validated diagnosis). We had some surprises when we performed autopsies from surgical departments and an important part of these patients were diagnosed with DCM as a primary or secondary diagnosis.

A quarter of all autopsies in which the diagnosis was DCM are from patients who died in the emergency room (ER).

Table 1

Differentiation between departments in County Hospital of Oradea during 2015 – 2016 in DCM dead patients

Patients discharged from departments												
Period	Total number of patients autopsied	Cardiology	Internal Medicine	General Surgery	Thoracic Surgery	Plastic Surgery	Urology	ENT	Gastroenterology	Neurology	Neurosurgery	ER
2015	62	8	17	6	1	1	0	1	6	7	1	14
2016	63	10	23	4	1	0	1	0	1	4	0	19
2015-2016	125	18	40	10	2	1	1	1	7	11	1	33

As seen in the literature, we meet in a lot of comorbidities especially pulmonary edema, atherosclerosis, stroke, hepatic impairment as shown in Table 2.

Table 2

The main comorbidities encountered in DCM patients (from autopsy reports)

Comorbidities		Number of patients affected in 2015	Number of patients affected in 2016	Total number of patients
Pulmonary	Edema	28	39	67
	Insufficiency	4	10	14
	Obstructive pulmonary disease	0	3	3
	Embolism	2	4	6
Hepatic	Chronic hepatitis	3	5	8
	Chronic vascular stasis	23	11	34
	Hepatomegaly	9	5	14
	Cardiac cirrhosis	0	3	3
	Hepatic cirrhosis	5	4	9
	Steatosis	7	9	16
	Insufficiency	1	1	2
Atherosclerosis		55	56	111
Stroke		9	7	16
Valvular damage	Aortic stenosis or insufficiency	11	10	21
	Mitral stenosis or insufficiency	8	6	14
	Tricuspid or	2	3	5

	pulmonary affection			

The most encountered comorbidity was atherosclerosis with different stages of evolution. In 8 corpses (from a total of 125) we didn`t find clear evidence of atherosclerosis.

Cardio-embolic comorbidities were encountered less than showed in the literature (1:5.68 had a fatal embolic event) and this happened because of strict thromboembolism prophylaxis in our center.

The majority of hepatic impairments were found in gross examination of the liver and on the cut surface. We have differentiated cardiac cirrhosis from hepatic cirrhosis based on production mechanism of these two end stage diseases.

Valvular damage was noticed in a few patients in our study and this shows that our examination technique is not the best in revealing these conditions.

Gross examination reveals global increased heart weight with a lesser consistency, dilation of all cavities and reducing the thickness of muscle. Endocardial fibrous thickening starts more often over the septal portion of the LV where there is a prominent fine reticulation of trabecular muscles. At the apex we can find mural thrombi. In final phases we can notice dilated aortic and mitral valve.

In microscopy, we followed more consistent the interstitial fibrosis and the deposition of collagen. Many histopathological changes appear especially in perivascular areas because of an increased activity of fibroblasts. Also this perivascular fibrosis is a hallmark of cardiosclerosis.

The pattern of fibrosis is diffuse or focal, with a predominant perivascular topography (Fig. 1). In interstitial fibrosis, it can be observed the presence of fibrillar collagen in intermuscular spaces (as noticed in Fig. 2). In areas with myocardial necrosis, we encounter increased interstitial fibrosis.

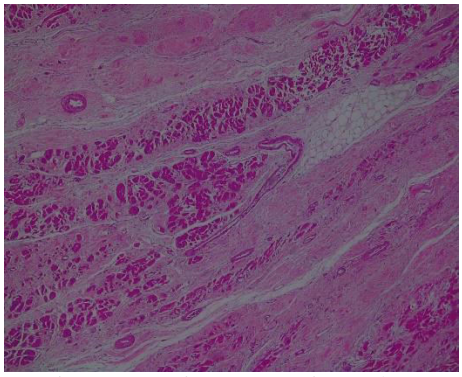

Fig.1: HE stain, 40X, perivascular and interstitial fibrosis in DCM

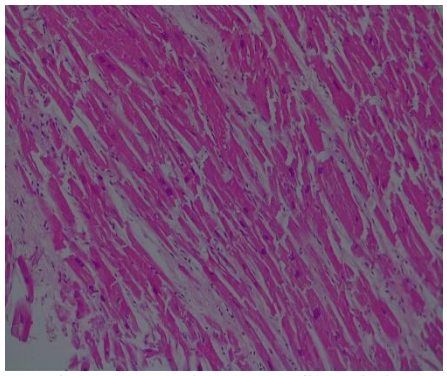

Fig. 2: HE stain, 100X, fibrosis in intermuscular spaces

In some cases, the distribution of the fibers was similar to those in ischemic heart disease (from smooth fibers to massive scars)

Collagen fibers were highlighted with polarized examination with shinny birefringence and are distributed individually (surrounding the cardiac fibers or in bundles (Fig.3). Coronary arteries have collagen accumulation in the wall that can be shown using special histochemical stains (trichrome Masson and trichrome Mallory) as shown in Fig. 4.

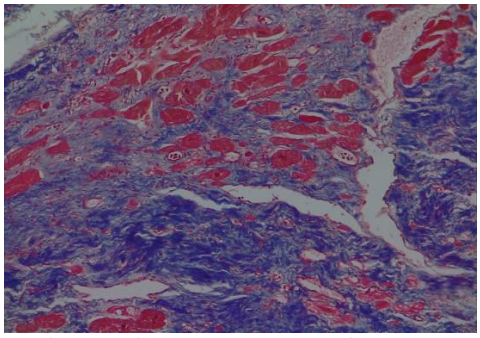

Fig. 3: Trichrome Masson stain, 100X, bundles of collagen fibers dissecting the cardiac muscle

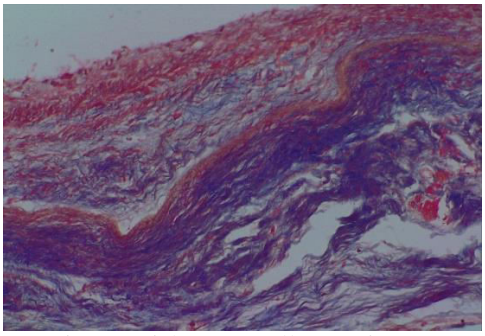

Fig. 4: Trichrome Mallory stain, 40X, vessel wall with collagen deposition

Also, in less affected areas we can appreciate the presence of rich anastomotic capillaries as shown to the normal control group.

DISCUSSION

The common features for all dilated cardiomyopathies are represented by a weak contraction, decrease of left ventricular wall thickness followed by a progressive expansion of the ventricular cavity. [Davies MJ,2000].

Familial dilated cardiomyopathy was proposed to be considered as a form of "cytoskeltopathy". [Bowles NE, Bowles KR, Towbin JA, 2000]

According to some authors, the most common cause of dilated cardiomyopathy is alcohol consumption. A wide range of structural abnormalities were observed in the myocardium, associated with high alcohol consumption, but is difficult to define precisely the point at which these abnormalities can be considered as being significant for dilated cardiomyopathy [Maron BJ, et al., 2006].

The intensity of histopathological alteration was with high variability from one patient to another and even from one area to another within the same specimen. Other authors [Hughes SE, WJ McKenna, 2005] have also

shown that histological changes associated with DCM are non-specific and not all may be present simultaneously.

The fibrosis may be due on one hand to myocardial hypoxia and on the other hand to the presence of large numbers of fibroblasts. It is known that, under hypoxic conditions, connective tissue cells have the ability to transform into fibroblasts (young connective cells) that can synthesize and excrete large amounts of connective matrix.

In our study, we found large amounts of collagen, arranged in bundles within the interstitial space that can impair the cardiac function in systole and in diastole because of the stiffness of muscle wall and the reduced oxygenation to cardiac fibers.

It is clear that interstitial fibrosis contributes to ventricular dysfunction and affects prognosis in patients with DCM [Soufen HN, et al., 2008].

Alterations in the extracellular matrix (ECM) result in cardiac fibrosis. Reactive and diffuse myocardial fibrosis is one of the main features of DCM. The most common localization of fibrosis in DCM is interstitial (pericellular).

The Masson's trichrome stain is particularly useful in the assessment of the main component of connective tissue e.g. collagen, which is the main component of interstitial fibrosis. In addition, myocardial fibrosis can be assessed indirectly by means of a wide array of imaging modalities. Although widely available, echocardiography and nuclear imaging have low specificity for ECM fibrosis detection, whereas positron emission tomography (PET) has high specificity but its availability is limited.

Therefore, cardiac magnetic resonance (CMR) is currently the method of choice for cardiac fibrosis assessment. CMR with delayed imaging following administration of gadolinium contrast allows the visualization of regional myocardial fibrosis via areas of late gadolinium enhancement (LGE). Moreover, assessment of diffuse fibrosis can be achieved with post-contrast enhanced T1 and T2 mapping. [Rubiś P, et al., 2017]

CONCLUSIONS

Cardiomyopathies are a major cause of morbidity and mortality in both children and adults.

The pathophysiology of dilated cardiomyopathy is heterogeneous both in terms of its pathogenesis and pathology.

DCM requires a strong financial support because of decompensated HF and are a frequent indication for heart transplantation.

In microscopy, changes of the interstitial fibrous tissue are sometimes extensive and sometimes barely noticeable. The most common

alteration of this structure was the onset and development of a mainly perivascular fibrous process and proliferation of collagen.

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