ORIGINAL ARTICLE

Histopathological and clinical aspects in dilated cardiomyopathies

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Abstract: Introduction – Dilated cardiomyopathy (DCM) represents a group of myocardial disorders associated with mechanical and electrical dysfunctions that causes enlargement of both ventricles. Over 50% of primary DCM are familial in nature and the rest have genetic etiology. Secondary DCM may occur during a wide range of diseases. Functionally, DCM is characterized by systolic dysfunction leading finally to a diminished left ventricular ejection fraction. A good correlation has been found between the severity of the condition clinically and the extent and degree of microscopic abnormalities. **Methods –** The macroscopic features can be best appreciated with a four-chamber view of the heart or by the short axis (bread loafing) slicing technique. Myocardial fragments are harvested, trimmed and kept in fixation solution for minimum 24 hours necessary for microscopic examination. Subsequently, specimens undergoes tissue processing. **Results –** Gross examination reveals increased heart weight with a globular shape and a decreased tonus. Endocardial fibrous thickening starts over the septal portion of the LV where there is a prominent fine reticulation of trabecular muscles. At microscopic level, different degrees of myofibrillar hypertrophy (enlarged, irregularly shaped muscle fibers with hyperchromatic, squared off nuclei) and collagenous fibrosis are detected. **Discussions –** Familial DCM is proposed to be considered as a form of "cytoskeltopathy". Interstitial fibrosis contributes to ventricular dysfunction and affects prognosis in patients with DCM. **Conclusions –** Without cardio-pulmonary transplant, patients with DCM has ominous prognosis with greatly reduced 5-year survival. The most common histologic alteration in DCM patients is the onset of perivascular fibrosis and proliferation of collagen fibers.

Keywords: dilated cardiomyopathy, histopathological examination, myocardial hypertrophy, endocardial fibrosis, cardio-pulmonary transplant

Rezumat: Introducere – Cardiomiopatia dilatativă (CMD) reunește un grup de afecțiuni miocardice asociate cu disfuncții mecanice și electrice care determină dilatație cavitară a ambilor ventriculi. Peste 50% din CMD primare au caracter familial, restul având o etiologie genetică. CMD secundară poate surveni în cursul unor boli infecțioase, endocrine, inflamatorii, pe fond toxic, în cadrul unor tezaurismoze, a unor tahiaritmii, hipotermii, iradieri. Din punct de vedere funcțional, CMD se caracterizează prin disfuncție sistolică ce evoluează, în final, spre reducerea fracției de ejecție a ventriculului stâng. O corelare între severitatea clinică a bolii și modificările microscopice a fost demonstrată. **Material și metodă –** Modificările macroscopice ale cordului pot fi apreciate cel mai bine printr-o secționare transversală a acestuia. Examinarea microscopică se efectuează prin recoltarea și fasonarea unui fragment de miocard ce necesită fixare de minim 24 ore în fixator universal care, ulterior va fi histoprocesat. **Rezultate –** La examenul necroptic, cordul prezintă o creștere în volum, o formă globuloasă și un tonus scăzut. La nivelul endocardului se evidențiază fibrozarea trabeculațiilor ce debutează la nivelul porțiunii septale a ventriculului stâng. La nivel microscopic se decelează grade diferite de hipertrofie miofibrilară și fibroză interstițială. **Discuții –** CMD familială este considerată o formă de „citoscheletopatie". Fibroza interstițială contribuie la disfuncția ventriculară și afectează prognosticul la acești pacienți. **Concluzii –** Fără un transplant cardiac, pacienții cu CMD evoluează infaust, supraviețuirea la 5 ani fiind mult redusă.

Cuvinte cheie: cardiomiopatie dilatativă, examen histopatologic, hipertrofie miocardică, fibroză endocardică, transplant cardio-pulmonar

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INTRODUCTION

Dilated cardiomyopathy (DCM) brings together a group of primary or secondary myocardial disorders that causes marked enlargement of both ventricles, diffuse parietal diskinesia and systolic dysfunction[1,2].

Although a universal definition of cardiomyopathy is not currently accepted, American Heart Association (AHA) defined in 2006 cardiomyopathies as „a heterogeneous group of myocardial diseases associated with mechanical and electrical dysfunctions that develops hypertrophy or myocardial dilation having many causes"[3].

Primary cardiomyopathies (DCM, hypertrophic cardiomyopathy, restrictive cardiomyopathy, arrhythmogenic cardiomyopathy, left ventricular noncompaction) are defined as inherent myocardial disorders and secondary ones occur in accordance with other diseases that affect myocardial function[4].

It is estimated that in the US, 5 to 6 million patients with heart failure were diagnosed, of which 5-10% had cardiomyopathy as substrate[2]. Also, DCM prevalence in the US is 36 cases / 100.000 inhabitants, with approximately 10.000 deaths per year1. The estimated European prevalence of DCM is 1: 2500 inhabitants[5].

Worldwide, DCM is known to be the most common cause of congestive heart failure in the adult population[4].

Over 50% of primary DCM are familial in nature (when seen in more than 2 family members), and the rest have genetic etiology (Duchenne muscle dystrophy, Friedreich ataxia, arhrythmogenic cardiomyopathy). Women who had recently gave birth can develop peripartum primary DCM[1,4].

Secondary DCM may occur during infectious diseases, endocrine disorders, inflammatory conditions, lysosomal storage disease, during the usage of toxic substances, in tachyarrhythmia, hypothermia, irradiation and others.

Secondary toxic DCM can be determined in particular by alcohol and cocaine. Other known cardio – toxic substances are: amphetamines, cobalt, lead, lithium, mercury, beryllium, metisergide. vitamin B1 and niacin deficiency also affects myocardial function[1].

Secondary endocrine based DCM is associated with thyroid disease, pheochromocytoma, Cushing's syndrome, diabetes mellitus and GH hypersecretion.

CMD may be induced by inflammatory conditions due to anti – myocardial antibodies: anti β-1 receptor antibodies, anti α-myosin heavy chain antibodies, anti β-myosin heavy chain antibodies, anti-small chain myosin antibodies and anti-troponin antibodies. Diseases that are associated with secondary DCM are: systemic lupus erythematosus, scleroderma, rheumatoid arthritis, dermatomyositis, giant cell arteritis (Horton›s disease), Kawasaki disease, sarcoidosis.

Iatrogenic DCM can be produced by a wide variety of drugs, especially chemotherapy (anthracyclines, transtuzumab, adriamycin) and anti-retroviral medication[1].

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[6].

The onset of the disease occurs most frequently in adulthood (20-60 years) with a mean age at presentation at about 50 years[7,8].

The main symptoms are progressive dyspnea, nocturnal paroxysmal dyspnea, decreased tolerance to physical activity, orthopnea and occasionally, patients may experience palpitations[1].

For diagnosis, electrocardiogram (ECG) can orient for etiology of DCM (coronary diseases, LV hypertrophy, amyloidosis, atrial fibrillation, and others). Thoracic radiography allows the evaluation of cardiomegaly, pleural effusions and pulmonary stasis[1].

Natremia, Kalemia, the levels of serum urea and creatinine are used to establish prognosis[9]. The most prominent stratification markers are BNP and NT - ProBNP.

Transthoracic echocardiography is required in all patients with new onset of heart failure and provides valuable data on cavity sizes, myocardial thickness, valvular and both ventricles function. In patients with DCM both ventricles are enlarged with diffuse hypokinesia of LV walls and systolic dysfunction. Echocardiography has a sensitivity regarding to reduced systolic function around 80% while specificity reaches 100%[9].

Stress ECG, angiography, multi-slice CT scan, magnetic resonance imaging can provide additional information in assessing DCM patients.

Endo - myocardial biopsy is indicated only in acute forms of CMD and eventually in post - myocardial treatment monitoring[1].

Genetic testing is considered useful in familial CMD forms detected by a screening of the first degree relatives of the patient[1].

About 30% of cases have an inherited basis, usually autosomal dominant, due to germline mutation in a

gene encoding sarcomeric protein, intermediate filament, nuclear membrane protein, cytoskeletal protein, phospholamban or ion channel protein[10].

The complex treatment of these patients is based on establishing the main principles: improvement in vital prognosis, symptoms, reduced morbidity and mortality. It is desirable to delay or reverse specific heart remodeling in heart failure. Treatment includes non-pharmacological methods, drugs, or implantable medical devices. The only chance of curative treatment of DCM patients is cardiac transplantation in very well-selected cases.

The prognostic factors used in the evaluation of DCM patients are: NYHA classes, LV ejection fraction, diastolic dysfunction, decrease of right ventricular function, decrease in O_2 maximum consumption, reduction of glomerular filtration rate, decrease in mean blood pressure[1].

However, the prognosis for patients with persistent DCM who do not undergo cardiac transplantation is poor with an average 5 – year survival rate under 50%[11].

A good correlation has been found between the severity of the condition clinically and the extent and degree of microscopic abnormalities, although the sometimes focal nature of the changes may be misleading[10].

METHODS

Our study included a total 63 deceased patients (from 1st of January 2016 until 31st of December 2016) which 44 were admitted in County Hospital of Oradea and in 19 cases death occurred in the emergency room. In all corpses, pathological autopsy was performed with the approval of Head of the Pathology Department. We excluded trauma related deaths. These procedures were carried out in accordance with the proper legislation[12].

Subjects were divided into age groups, differentiation between sex, environment and main comorbidities. All the data was processed using Microsoft Excel and SPSS Statistics 21.

For the autopsy technique, we used Rokitansky method that implies examining the organs with maintaining connections and anatomical relations with nearby structures.

After opening the thorax by removing the sternum, the cervico – thoracic organs (tongue, larynx, bronchopulmonary piece, the heart with large vessels, esophagus, thyroid and the anterior mediastinum) are taken in one piece.

The pericardium is opened using a sharp pointed scissors and the heart is highlighted with the atrial auricles and the base (removing pericardial tissue and fat will expose the great vessels).

The macroscopic features can be best appreciated with a four-chamber view of the heart or by the short axis (bread loafing) slicing technique. We sliced the heart at 1 cm intervals in the short axis (horizontal plane) at the apex and continuing to just below the inferior margin of the atrioventricular valve leaflets. Next, we open the atrial and the remaining portions of the ventricular chambers. After removing the vessels and residual postmortem blood clots the heart is being weighed[13].

Myocardial fragments are harvested, trimmed and kept in fixation solution (10% buffered formalin) for

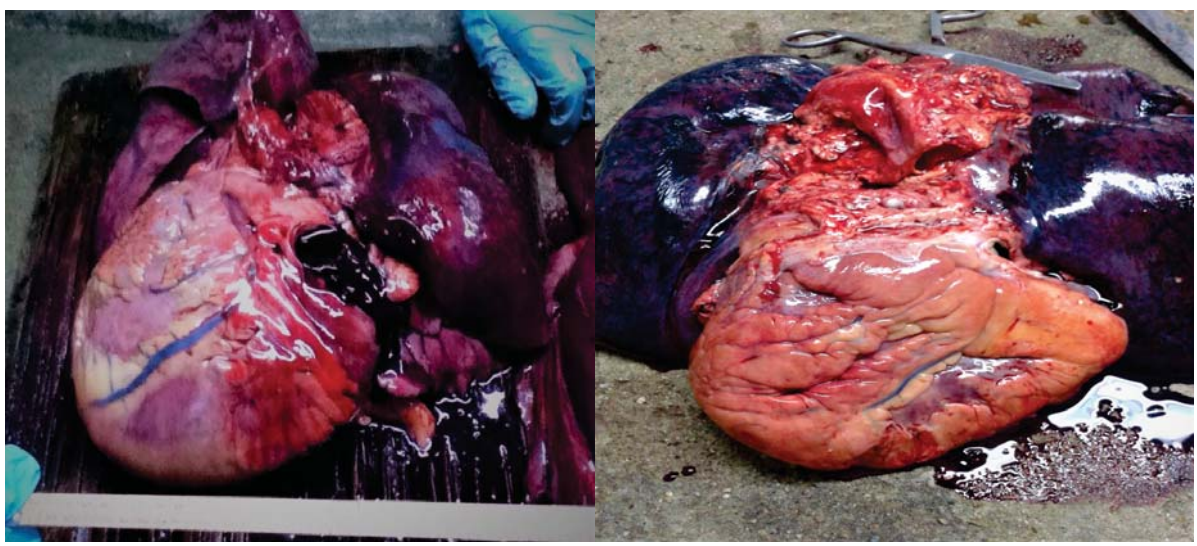

Figure 1. Gross appearances of increased in volume hearts in DCM.

minimum 24 hours necessary for microscopic examination. Subsequently, the fragments were being processed using a Leica TP1020 automatic tissue processor. After processing, the tissue is included in a paraffin block and sectioned at a thickness of 3 to 5 µm using a Leica manual microtome. The sections are displayed on slides, for drying and de – waxing. The slides are stained using Hematoxylin – Eosin, a special stain for collagen fibers (Masson's trichrome) and then we mount a coverslip. 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.

RESULTS

From all 63 cases autopsied with DCM diagnosis, most of them were admitted to Cardiology and Internal Medicine Department (33 corpses). In the Emergency Room 19 patients had DCM in the tanatogenesis of irreversible cardiac arrest. A number of 21 cases had DCM as the main death diagnosis, while cardiomegaly was detected as a comorbidity in 42 patients.

In 6 autopsies performed on female patients, DCM was the main cause of death, despite of 20 body examination, when DCM was an associate diagnosis, in the same gender.

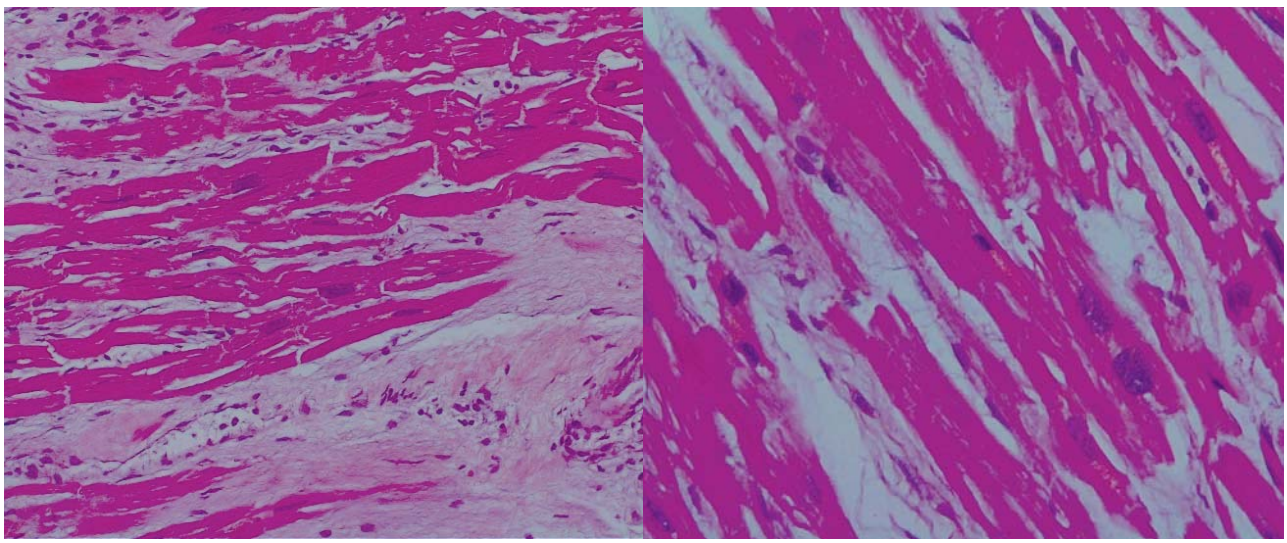

Figure 2. Hematoxylin Eosin stain, hypertrophic myocardial fibers with „box car" nuclei, fibroblasts and interstitial fibrosis.

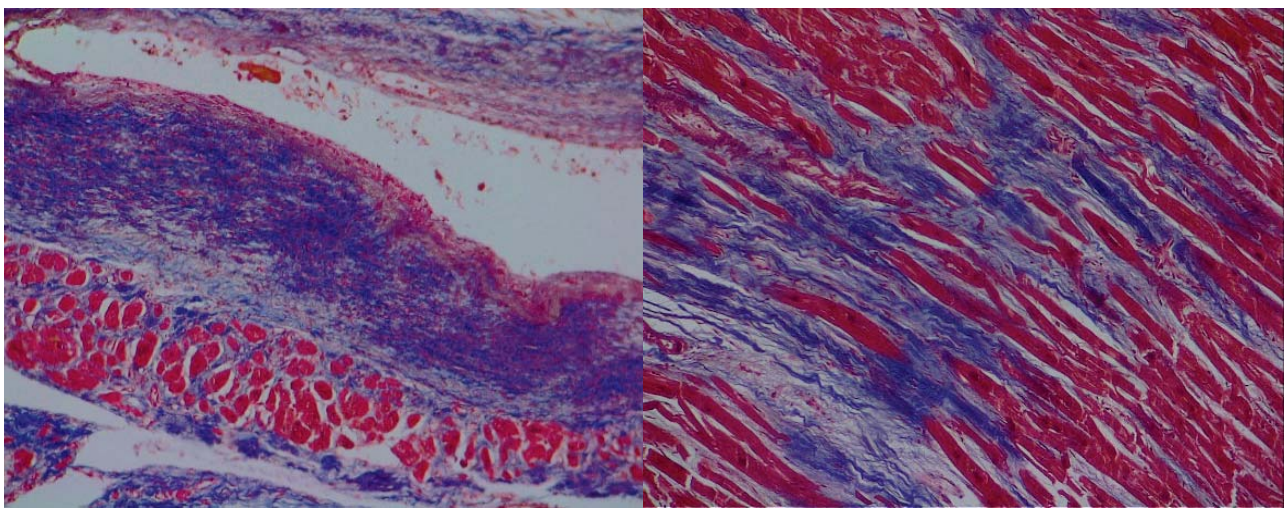

Figure 3. Masson's trichrome stain showing subendocardial scars and interstitial deposition of collagen fibers.

Total number of men with DCM as a primary diagnosis was 15, while in 22 autopsies, DCM was found as a comorbidity. In this gender, alcoholic etiology of DCM was suspected in 11 patients that had alcoholic liver cirrhosis and steato – hepatitis without virus implication.

In 6 corpses, clear signs of atherosclerosis were not found and in these patients and we excluded ischemic etiology of DCM. In 17 cases DCM had a valvular background and in 9 patients autopsied, we didn`t found signs of secondary DCM. These cases had a final diagnosis of primary DCM. No peripartum DCM was described in our cohort.

In autopsied patients, mean age was 66,47 years (n = 63) with a standard deviation of 13.83 years. Mean age of patients admitted in Cardiology Department was 67.7 years old (n = 10) with a standard deviation of 10.15 years that was similar with the age of all admitted patients from our hospital (66,31 years old with a standard deviation of 13.80 years in 44 corpses).

By background, we didn't saw a differentiation between patients from cities or rural sites (30 subjects were form urban regions and 33 came to hospital from countryside).

Gross examination reveals increased heart weight with a globular shape and a decreased tonus. On section, in the initial phases, an adaptive increase in myocardial thickness is observed (this muscle hypertrophy is a response to intracavitary pressure). Subsequently, excessive cavity dilatation begins simultaneous with myocardial thinning and may appear as „bovine heart". Endocardium changes color, becoming greyish with trabecular fibrosis and stiffness. Endocardial fibrous thickening starts over the septal portion of the LV where there is a prominent fine reticulation of trabecular muscles. The thin myocardium presents diffuse discolorations, fine whitish streaks, some of which are branched, with maximal dimensions of up to 5 mm. Intracavitary we can find mural thrombi, especially at apical level. Annular dilated atrioventricular valves appear in the final phases of DCM, which confirms valvular regurgitation diagnosed during life.

At microscopic level, different degrees of myocyte hypertrophy (enlarged, irregularly shaped muscle fibers with hyperchromatic, squared off nuclei) and collagenous fibrosis are detected. Fibrosis may extend into subendocardial layers, may be diffuse or perivascular. Interstitial cellularity is represented by fibrous cells, fibroblasts and possibly lymphocytes (especially in secondary DCM, postmyocarditis). Some myocardial fibers may exhibit degenerative changes and a perinuclear attrition pigment (lipofuscin).

Two important details for differential diagnosis in endomyocardial biopsies are leukocytic infiltrates (is present in about one-half of all patients with myocarditis) and the absence of myofibrillar lesions.

DISCUSSIONS

Although the onset of DCM occurs with a mean age at presentation at about 50 years, in our study the mean age of death in patients with DCM was 66.47 years old (n = 63)[7,8].

Even if we diagnose patients at a younger age, mortality rate is higher in elderly patients because of the effectiveness of new cardiac remodeling therapies. The 5-year mortality of patients with non-ischemic CMD is high, with 20% of them having sudden cardiac death. Familial DCM is proposed to be considered as a form of „cytoskeltopathy"[14].

A correlation has been found between severity of the clinical signs and the severity of microscopic lesions, although the sometimes focal extent of changes may be misleading[10].

Inflammation is an adaptive response to cardiac injury, but the role of a prolonged inflammatory response has been well established as maladaptive heart structural alteration. Evidence for cell-mediated immunity includes involvement of pro – inflammatory cytokines and lymphocytic infiltration on biopsies in almost half of patients with idiopathic DCM[15].

It is clear that interstitial fibrosis contributes to ventricular dysfunction and affects prognosis in patients with DCM[16].

CONCLUSIONS

DCM is known to be the most common cause of congestive heart failure. Without cardio-pulmonary transplant, patients with DCM has ominous prognosis with greatly reduced 5-year survival.

Early detection and proper treatment may improve the quality of life in these patients.

The most common structural heart alteration in DCM patients is the development of perivascular fibrosis and proliferation of collagen fibers.

Conflict of interest: none declared.

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