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Changes of desmin expression pattern in the myocardium of patients with alcoholic dilated cardiomyopathy

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Abstract

Introduction: It has been suggested that desmin cytoskeleton remodeling may contribute to the progression of dilated cardiomyopathy and might affect long-term prognosis. This study is aiming at evaluating desmin expression in cardiomyocytes from patients with dilated cardiomyopathy of alcoholic etiology in advanced stages of the disease and comparing the results with measurements of normal heart tissue from control patients. *Materials and Methods*: For immunohistochemistry, sections from 36 myocardium fragments taken from left ventricle of dilated cardiomyopathy patients were immunolabeled with an anti-desmin antibody and negative control slides were obtained by omitting the primary antibody. We calculated the ratios between the areas of myocardiocytes and the length and number of A dark disks and assessed the desmin expression level as the integrated optical density (IOD) and, respectively, the total areas of the signal given by immunolabeling. A Student's *t*-test has been utilized to assess the differences, $p<0.05$ deemed significant data. *Results*: We identified significant decrease in numerical density of dark disks in our cases group compared with controls ($p<0.05$). Also, the ratios between total cellular area and total length of dark disks and number of dark disks was significantly different between cases and controls ($p=0.04$). IOD was significantly different between dilative cardiomyopathy cases and controls and also, overall desmin expression area was increased in dilatative cardiomyopathy patients. *Conclusions*: The identification of different desmin expression and standardization in diseased myocardium may be helpful in stratifying patients and in understanding their evolution, but also in finding new therapeutic targets that aim the alterations in desmin expression.

Keywords: dilated cardiomyopathy, desmin expression, integrated optical density, sarcomeric disks.

Introduction

Dilated cardiomyopathy is a primary myocardial disorder characterized by ventricular dilatation and impaired systolic function, making it a common cause of heart failure and the leading indication for heart transplant. The prevalence of the disease estimated in a prospective post-mortem study since 1997 is 1:2500 and the incidence of cases discovered at autopsy is 4.5:100 000/year [1].

Heart tissue in general and cardiomyocytes in particular have a high degree of organization since their functional role needs to be at a maximal level. Any kind of mutation occurred in cytoskeletal proteins can have devastating effects on the architecture of the heart tissue and consequently on its functionality. In dilated cardiomyopathy, while tissue changes are resumed by areas of fibrosis and regional myocyte dropout, the changes in cytoarchitecture are occurring usually at the level of intercalated disks, affecting different components. Wilson *et al.* stated in their study that newly formed sarcomeres have a disorganized attachment to intercalated disks [2]. Also, it was found that in murine heart muscle affected by dilated cardiomyopathy there is a low expression of connexin-43, which leads to fewer gap junctions between cells, therefore a weaker intercellular communication [3]. These alterations were also found in human heart tissue, though less penetrant [4].

The role of desmin and its associated proteins in biogenesis and function of organelles and their implication in the development, differentiation and survival of heart muscle cells are described in different studies, but the exact mechanisms remain elusive [5].

Desmin plays an essential role in the cell, it maintains the architecture of all types of muscle cells, it intervenes in the process of contraction of the cell by binding the sarcomeric contractile apparatus to subsarcolemmal cytoskeleton and nuclear and mitochondrial membrane [6]. Desmin is abundant in intercalated disks, and due to its interconnections, it integrates the contractile function of myofilaments. Targeted gene studies indicate that desmin is not required in embryonic myogenesis, but later on is essential for maintaining the structural and functional integrity of myocytes.

Desmin expression in cardiac pathology is often down-regulated [7] and it is one of the basic structural, muscle specific proteins, which is believed to play an important role in the progression of heart failure [8]. It has been suggested that desmin cytoskeleton remodeling may contribute to the progression of dilated cardiomyopathy and might affect long-term prognosis. Recent studies

report that in patients with dilated cardiomyopathy, decreased desmin expression or lack of it seems to be a strong independent predictor of unfavorable prognosis [9].

This study is aiming at evaluating desmin expression in cardiomyocytes from patients with dilated cardiomyopathy of alcoholic etiology in advanced stages of the disease and comparing the results with measurements of normal heart tissue from control patients.

Materials and Methods

This study was performed on a number of 36 hearts from patients deceased with dilated cardiomyopathy of alcoholic etiology and to whom the necropsies were performed in the Department of Pathology from the Emergency County Hospital, Craiova, Romania. From the hearts prelevated during the autopsies, we have obtained myocardium fragments from the left ventricle, fragments which have been further fixed in neutral buffered formalin and then processed for paraffin embedding. As control, we used normal myocardial tissue taken from 12 patients who died from non-cardiac causes (stroke, cancer, etc.).

Routine Hematoxylin–Eosin (HE), Periodic Acid–Schiff (PAS) and Goldner–Szekely trichrome stainings have been utilized on 4 μm-thick sections, as primary basic stainings for diagnostic purposes.

For immunohistochemistry, consecutive tissue sections were deparaffinized, rehydrated in graded alcohol series, and processed for immunolabeling with an anti-desmin antibody utilizing a Bond-Max Leica immunostainer with its proprietary polymer-amplification and 3,3'-Diaminobenzidine (DAB) detection systems. All slides were processed with the same protocol settings for consistency, together with negative control slides, which were obtained by omitting the primary antibody.

Random images have been taken with a 40× objective on a Nikon Eclipse 55i microscope (Nikon Instruments Europe BV, Amsterdam, The Netherlands) equipped with a 5 Mp charge-coupled device (CCD) camera and the image processing software Image ProPlus AMS (Media Cybernetics, Bethesda, MA, USA). After the pixel-size calibration, we have measured directly on the slides the areas of individual myocardiocytes (between consecutive intercalated disks), as well as the length and number of A dark bands, as stained by desmin. For each slide, we have next calculated the total myocardiocyte areas and divided them by either the total A disks length or number. Results have been reported as average ± standard deviation (SD), and Student's *t*-test has been utilized further on to identify statistically significant differences. Correlations between numerical data groups were sought utilizing the Pearson's correlation coefficient. $P<0.05$ was utilized to deem significant data. All data has been evaluated and analyzed in Microsoft Excel and IBM's Statistical Package for the Social Sciences (SPSS).

Results

Histopathological analysis of the 36 selected cases from deceased patients with the diagnosis of dilated cardiomyopathy of alcoholic etiology revealed a number of changes in cardiomyocyte structure and in the extracellular matrix. There were differences between cardiomyocytes regarding their size and shape, a loss of myofibrils was identifiable together with the presence of intracellular spaces in which there was an amorphous, basophil, PAS-positive material accumulated. Nuclear pleomorphism was also found (Figures 1 and 2).

In the extracellular matrix, we found collagen fibrosis, which constantly surrounded cardiomyocytes (Figure 3).

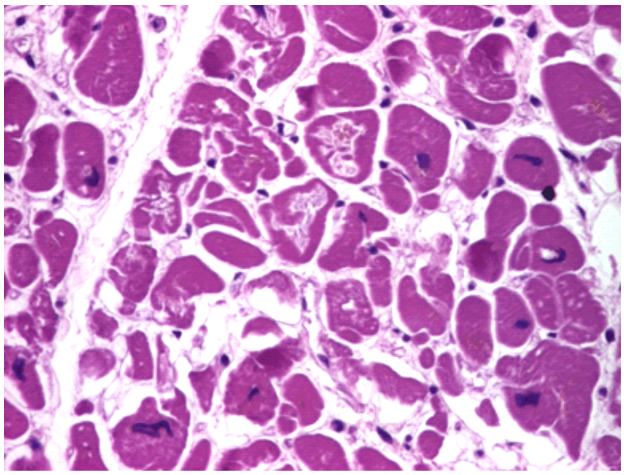

Figure 1 – ***Cardiomyocytes: variations of their size and shape, loss of myofibrils. Cytoplasmic accumulation of amorphous, basophilic material. Nuclear pleomorphism (HE staining, ×200).***

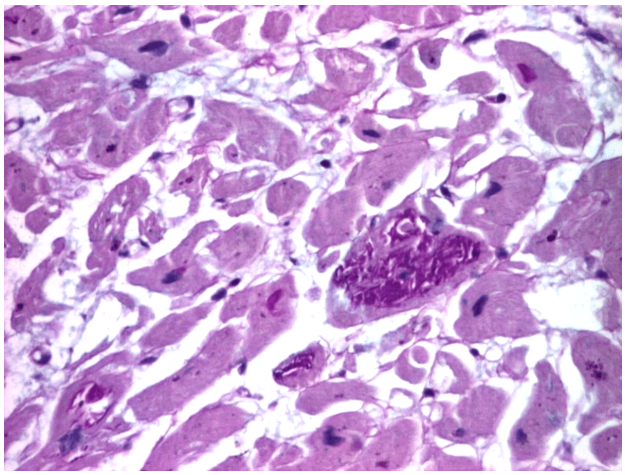

Figure 2 – ***Cytoplasmic accumulation of PAS-positive material (PAS staining, ×200).***

The cases investigated had revealed the heterogeneity in desmin immunoexpression, highlighted by the alternation between areas with preserved desmin expression and areas in which the expression was absent (Figure 4). The loss of desmin expression had a centripetal pattern starting in perinuclear cytoplasmic areas and with a relative preservation near the intercalated disks (Figure 5). On occasion, desmin reactivity showed a marked reduction in some myocardiocytes with apparent preservation in immediately adjacent cells (Figure 6), and in other instances, the linear-like reactivity pattern along the dark disks was lost in favor of a more diffuse and dotty-like disorganized staining (Figure 7).

Based on the expression of desmin in myocardiocytes,

we were able to identify a significant decrease in the numerical density of dark disks in cases of dilated cardiomyopathy compared with control cases, $p<0.05$. There was a significant difference between the selected cases (1.94±0.31) and controls (1.824±0.13) in what it regards the ratio between the total measured cellular area and the total length of the dark disks ($p=0.041$) (Figure 8A). Also, the ratios between total cellular areas and the number of dark disks was significantly different between patients with dilated cardiomyopathy (17.98±5.891) and controls (12.449±2.654), $p<0.001$ (Figure 8B).

We next assessed the desmin expression level as the integrated optical density (IOD) and respectively, the areas of the signal given by desmin immunohistochemistry. IOD was significantly different between dilative cardiomyopathy cases (563 40.1±320 689.59) and controls (415 966.76± 213 112.85), $p=0.0283$ (Figure 9A). Also, overall desmin expression area was increased in dilative cardiomyopathy patients (4496.89±2650.61 μm^2) compared to control hearts (3413.93±1721.07 μm^2), $p=0.047$ (Figure 9B).

As expected, there was a strong correlation between signal IOD and area ($r=0.99$, $p<0.0001$) for both control and pathological cases (data not showed), as the IOD includes area in its formulation.

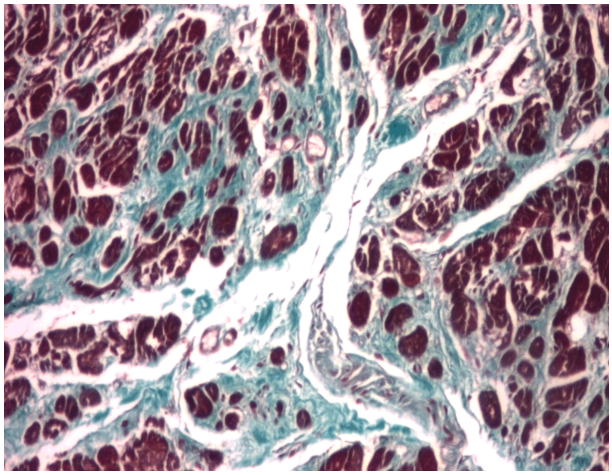

Figure 3 – ***Cardiomyocytes surrounded by fibrosis (Goldner–Szekely trichrome staining, ×100).***

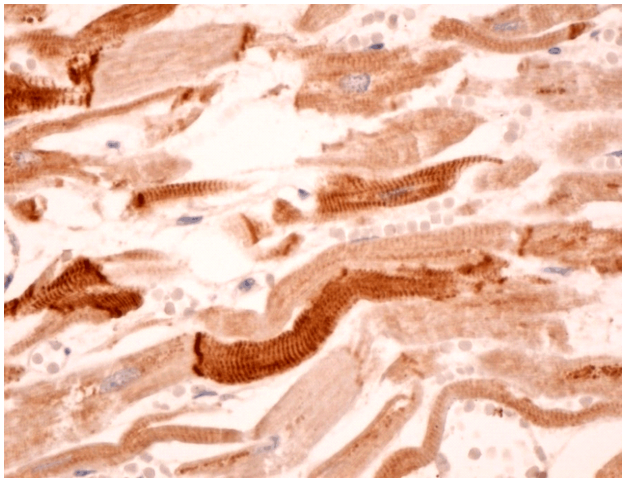

Figure 4 – ***Loss of myofibrils reactivity for desmin, with positive isolated cells surrounded by non-reactive cells (Desmin immunostaining, ×200).***

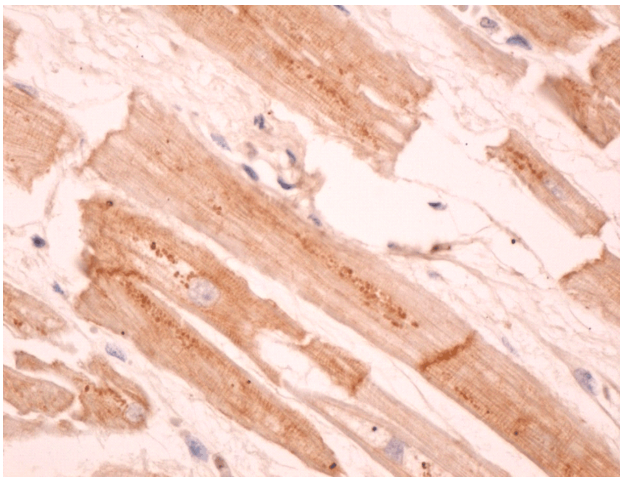

Figure 5 – ***Loss of myofibrils and lipofuscin deposits in cardiac myocytes. Collagen fibrosis surrounding the myocytes (Desmin immunostaining, ×200).***

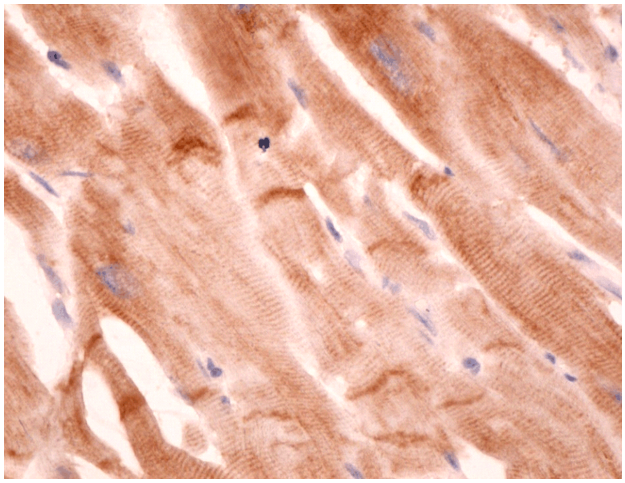

Figure 6 – ***Reduced cytoplasmic myofibrils reactivity for desmin with preserved immunoreactivity at the level of the intercalated disks (Desmin immunostaining, ×200).***

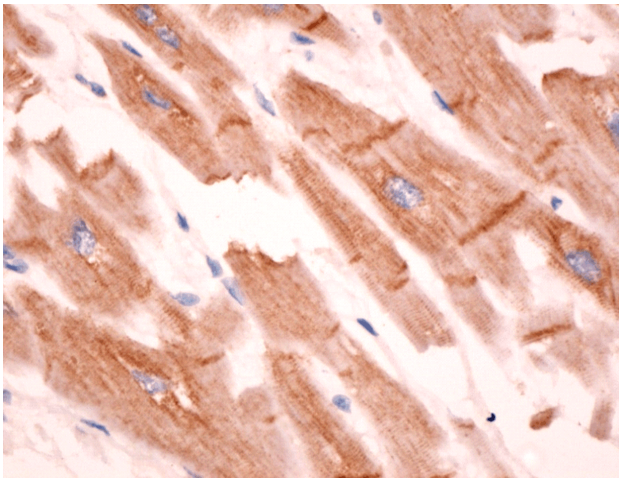

Figure 7 – ***Disorganization (fragmentation and irregularity) of cytoplasmic myofibrils reactivity for desmin with preserved immunoreactivity at the level of the intercalated disks (Desmin immunostaining, ×200).***

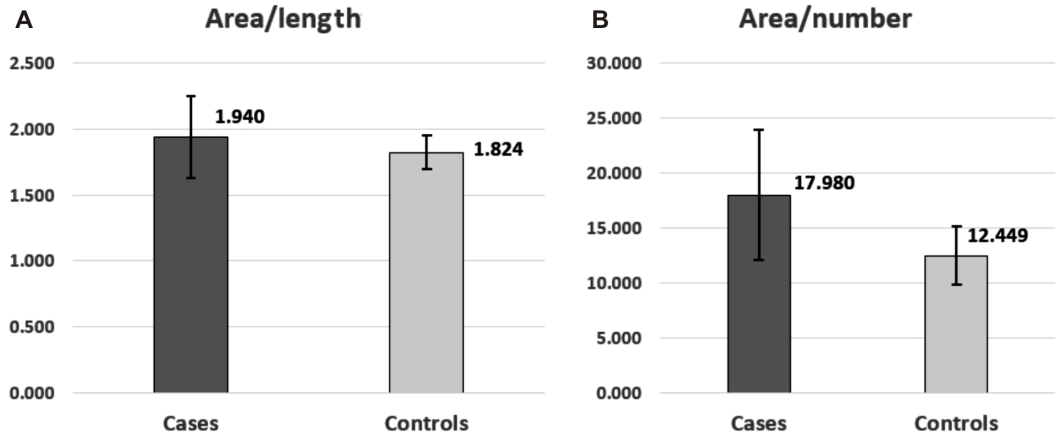

Figure 8 – ***(A and B) Averages of the length and number of dark disks (as stained for desmin) are illustrated here when normalized for the total myocardiocyte areas, using the Student's t-testing. Error bars represent standard deviation of the means.***

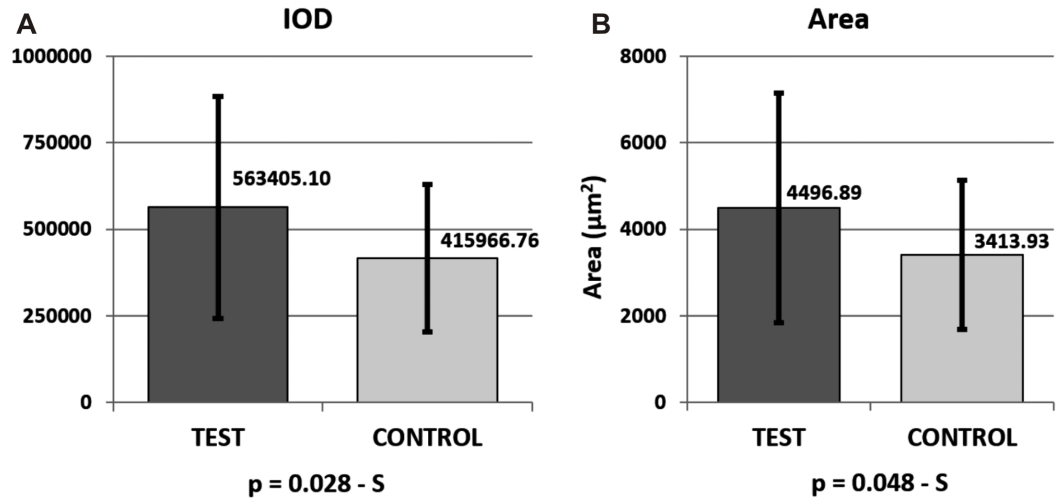

Figure 9 – ***Desmin average immunoreactivity is different between cases and controls in what it regards both the integrated optical density (IOD) (A) and the area (B), using the Student's t-testing. Error bars represent standard deviation of the means.***

We have also looked at the dependence between the number of dark disks and their average length, and while there was a strong correlation for control cases (r=0.67, p<0.0001), there was no correlation for cardiomyopathy cases (r=0.22, p=0.074) (Figure 10). Lastly, we have also checked for any correlation between area/length index and the IOD, and found that while this time there was no correlation for control cases (r=0.23, p=0.142), there was a moderate but significant correlation for the pathological counterpart (r=0.3, p=0.023) (Figure 11).

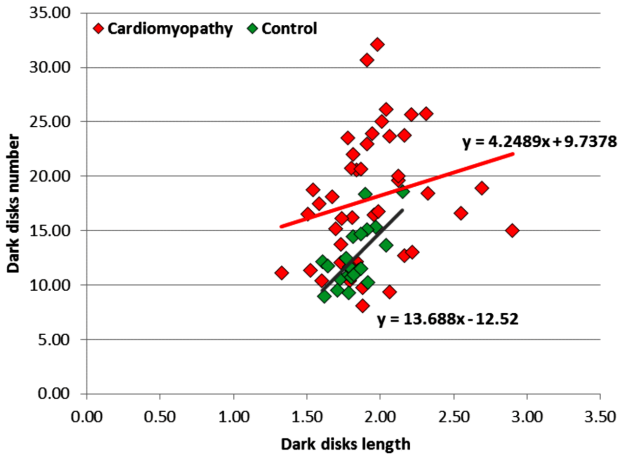

Figure 10 – ***Correlations between ratios area/number of dark disks and area/length of dark disks have yield a statististical significance only for control cases according to Pearson's coefficient (r=0.67, p<0.0001).***

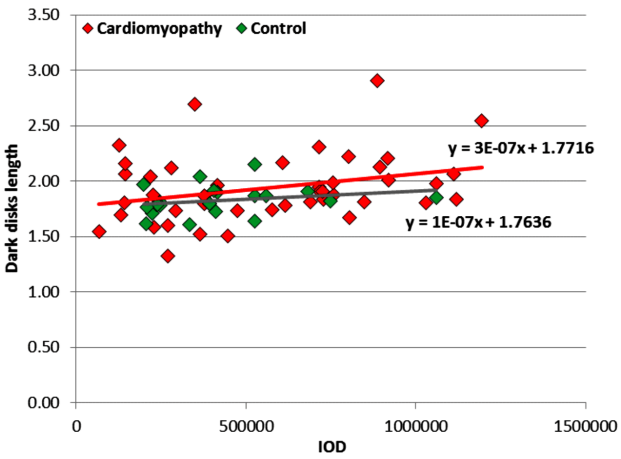

Figure 11 – ***Correlations between integrated optical density (IOD) and area/length ratio reaches statistical significance only for the pathological cases (r=0.30, p=0.023).***

Discussion

The histopathological diagnosis of dilated cardiomyopathy is one of exclusion, the lesions identified are common to other causes of heart failure. Pathological changes cited in the literature for this disorder are non-specific and are similar to those identified by the present study.

In patients with dilated cardiomyopathy immunolocalization of cytoskeletal proteins revealed their abnormal distribution. A study in this regard had shown an increase in the expression of desmin and its uneven distribution inside the cells [10]. Desmin is a 53-kDa subunit of Class III muscle specific intermediate filaments, which plays a crucial role in the maturation, maintenance and restoration of muscle fibers by forming interconnections around myofibrils, which are then connected to the sarcolemma and nuclear membrane [11–14]. The filaments of desmin in cardiomyocytes surround myofibrils and *Z*-bands and binds them to each other and to the cell membrane, forming a very dense network [15, 16]. Moreover, desmin builds a fine network linking the nucleus with mitochondria and endoplasmic reticulum.

Desmin function in cardiomyocytes is still quite unclear [8]. Several studies suggest that desmin may be involved in regulating gene expression, myofibrillogenesis and in intercellular signaling pathways [11, 17, 18], and may also be responsible for the shape of the cell membrane and of the cytoplasmic organelles [19, 20].

Compared with healthy individuals, in patients with heart failure was observed an altered expression of desmin, which may be associated with abnormal structure of the cells, lesion localization and functional alterations [8]. Some data suggest that cardiac dysfunction is linked to the intracellular expression of desmin [21]. Researchers have found that several cardiomyocyte abnormalities are linked to altered expression of desmin and to the forming of aggregates inside the cells [22]. Also, studies using transgenic mice have demonstrated that complex processes like remodeling of the heart and cardiac dysfunction are linked to disorganization and altered distribution of desmin filaments [23, 24].

Another study found that the immunohistochemical expression of desmin correlates with the extent of myocardial damage at the intracellular level [9]. Thus, it has been shown that desmin level begins to lower considerably in 30 minutes after acute ischemia and is almost absent after 90–120 minutes of myocardial infarction [25].

Myocardial tissue of end-stage heart failure patients had been shown to have a low number of desmin-positive myocytes compared with normal individuals, suggesting that desmin expression is associated with reduced heart function [26].

Some studies have suggested that patients with abnormal levels (excess or deficit) of desmin in cardiomyocytes had a significantly more unfavorable clinical prognosis than patients with normal levels of desmin [27]. The progression of cardiac disease leads to the development of compensatory reactions in order to maintain cell function, increased expression of desmin being one of these compensatory factors. If the disease progression cannot be stopped, compensatory mechanisms that lower cellular levels of desmin are triggered [28]. As a result, in cases of chronic severe heart failure, desmin expression in cardiomyocytes is reduced or absent compared to normal individuals, phenomenon intensified by the increase in size of cardiomyocytes [8].

This study aimed to evaluate the extent of myocardial injury and intensity of desmin expression in cardiomyocytes from patients with dilated cardiomyopathy of alcoholic etiology. Morphometry study revealed that, though the length of the dark disks is not significantly different between cases and controls studied, the overall density of desmin expression in cardiomyocytes was significantly lower in cardiomyopathy cases than in controls. These findings are in accordance with results from several studies on the subject (mentioned above) which revealed that in advanced stages of the disease, desmin cardiomyocyte expression is lowered as a result of the compensatory mechanism of the cells and their increase in size.

Also, desmin expression was evaluated by densitometry in the present study, which is related to antigen concentration and our results showed significantly higher levels of desmin density in cases of dilated cardiomyopathy compared to controls. Di Somma *et al.* [29] showed in their study about intermediate filaments in dilated cardiomyopathy that optical density of desmin is higher in these cases compared to controls and this increase was in linear relation to myocyte diameter. Thus, in dilated cardiomyopathy cells, the density of desmin is higher than in controls but the distribution has a centripetal, more irregular pattern, with accumulation in perinuclear areas. In a study published in 2013 by Pawlak *et al.* [9], desmin expression was categorized in four types of staining, type I, in which the staining was normal, at *Z*-lines and intercalated disks, type IIA, type IIB and type III in which desmin staining was diminished or absent. These types of desmin staining were correlated with survival of the patients. Thus, the worst survival rate was found in patients with type III expression of desmin compared to the other types of expression. Also, lack or decreased desmin expression was correlated with the lowest left ventricular ejection fraction, the highest levels of N-terminal prohormone of brain natriuretic peptide (NT-proBNP) and greater left ventricle dimensions. The results in the same study also pointed out that desmin expression was a better predictor of mortality than *New York Heart Association* (NYHA) class in this category of patients. In our study, there was a paralleled increase in the number and length of the dark disks for control cases, as highlighted by their direct correlation, while there did not seem to be a significant correlation for the patients with dilated cardiomyopathy, this suggesting a heretical increase in their number and length, with the loss of the normal balance of an average dark disk length. However, the total length of the dark disks did correlate with the desmin optical density for the pathological state,

while it seems to be a constant balance and thus no correlation for control myocardiocytes.

Also, the main findings of this study showed that abnormal expression pattern of myocyte desmin was found in 70% of dilated cardiomyopathy cases and it was correlated with survival rate and that desmin expression pattern is an independent prognostic factor related to poorer outcomes in patients with dilated cardiomyopathy [9].

Monreal *et al.* [30] found that changes in desmin expression occur not only in ischemic conditions, but also in non-ischemic conditions, suggesting that mechanical stress may be the causal factor for this type of changes. This study also concluded that desmin expression is better correlated with cardiac dysfunction than fibrosis or hypertrophy.

Conclusions

Desmin expression alterations play an important role in the functionality of the heart. There are important correlations between desmin expression alteration and outcomes, functional status and prognosis while it has been established the strong correlation between desmin expression and myocardial injury stratification and also the correlation with key features of chronic heart failure like echocardiographic, biological and clinical parameters. The identification of different desmin expression patterns and their standardization in diseased myocardium may be helpful in stratifying patients and in understanding the evolution of the disease, but also in finding new therapeutic targets that aim the alterations in desmin expression.

Conflict of interests

The authors declare that they have no conflict of interests.

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