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ORIGINAL ARTICLE

Evaluation of Galectin-3 and Myocardial Fibrosis in Patients with Hypertrophic Cardiomyopathy

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Abstract

Background: Galectin-3 is the designation given to the protein that binds to ß-galactosides, expressed by activated macrophages and described as a cardiac fibrosis mediator. In hypertrophic cardiomyopathy (HCM), myocardial fibrosis is an independent predictor of adverse outcome; however, the association between Galectin-3 and myocardial fibrosis has not been studied in this cardiopathy.

Objective: To evaluate the association of Galectin-3 and the presence of myocardial fibrosis in a patient with hypertrophic cardiomyopathy.

Methods: Galectin-3 was measured in automated equipment using the Elisa technique in 100 participants divided into two groups: 50 patients with hypertrophic cardiomyopathy and 50 healthy control subjects. All patients with hypertrophic cardiomyopathy underwent magnetic nuclear resonance with the late enhancement technique to investigate myocardial fibrosis. For the statistical analysis, p values < 0.05 were considered statistically significant.

Results: Galectin-3 levels were low and did not show significant differences between patients with hypertrophic cardiomyopathy and the control group, 10.3 ± 3.1 ng/dL and 11.3 ± 2.6 ng/dL (p = 0.12) respectively. Myocardial fibrosis was a common finding and was identified in 84% (42/50) of patients with HCM, but no differences were observed between Galectin-3 levels when comparing patients with and without fibrosis, 10.3 ± 2.4 ng/dL and 10.1 ± 2.1 ng/dL (p = 0.59).

Conclusion: The results did not show an association between Galectin-3 and myocardial fibrosis in patients with hypertrophic cardiomyopathy, suggesting that non-inflammatory mechanisms of myocardial fibrosis formation and cardiac remodeling are involved in this cardiopathy. (Int J Cardiovasc Sci. 2019;32(2)152-157)

Keywords: Cardiomyopathy, Hypertrophic; Endomyocardial Fibrosis; Galectin 3; Diagnostic Imaging; Arrhythmias, Cardiac.

Introduction

Hypertrophic cardiomyopathy (HCM) is defined by the presence of ventricular hypertrophy in the absence of cardiac or systemic diseases that justify the development of this muscular alteration.[1] It is the most common cardiopathy with a genetic cause, with an estimated prevalence of 0.2% (1:500).[2,3] Histologically, in addition to the hypertrophy and myocyte architecture disarray, varied increase in interstitial collagen and myocardial fibrosis occurs.[4] Recent studies showed that fibrosis presence and extent are an independent predictor for the occurrence of sudden cardiac death (SCD) and progression to heart failure (HF) in these individuals.[5-9]

Currently, Galectin-3 (Gal-3), a protein of the lectin family, has emerged as a new biomarker associated with myocardial fibrosis in acute and chronic heart failure (HF).[10,11] In the presence of inflammation, Gal-3 is secreted by activated macrophages, having the cardiac fibroblasts as binding sites, resulting in increased

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myocardial collagen expression and interstitial fibrosis.[12,13] Recent studies demonstrate the association between Gal-3 with inflammation and fibrosis, and these pathophysiological processes are related to adverse cardiac remodeling in HF.[14-16]

The aim of the present study was to evaluate Gal-3 values in patients with HCM and their association to myocardial fibrosis.

Method

Study population

Gal-3 plasma concentrations were analyzed in 100 subjects divided into two groups: 50 patients with a diagnosis of HCM and 50 healthy control subjects, whose clinical history showed no previous comorbidities, cardiovascular diseases, neoplasms or continuous medication use. The sampling was carried out by convenience.

Patients were included consecutively. The inclusion criteria were individuals of both genders, aged between 15 and 65 years, with a previous diagnosis of HCM, established by echocardiography (parietal thickness > 15 mm in any LV segment or ≥ 13 mm in patients with first-degree relatives who had the disease without cavity dilatation and in the absence of any other cardiac or systemic condition that could be the cause of hypertrophy). Exclusion criteria were: known kidney disease (serum creatinine > 2.0 mg/dL), individuals with hepatic cirrhosis of any etiology, patients with kidney or pulmonary fibrosis.

All patients with HCM underwent cardiac magnetic resonance imaging (CMRI) aiming at evaluating the presence of myocardial fibrosis detected by the late gadolinium enhancement (LGE) technique.

The study was approved by the medical ethics committee of the hospital and performed in accordance with the Declaration of Helsinki. Free and informed consent was obtained in written form from all patients.

Galectin-3 measurement

Galectin-3 measurements were performed in blood samples, collected after overnight fasting, centrifuged and immediately stored at -80°C. The analysis was performed using the Enzyme-linked Fluorescence Assay (ELFA) (Biomerieux, Marcy-l'Étoile, LY-France). Assay calibration was performed according to the manufacturer's recommendations. Gal-3 values ≤ 17.8 ng/dL were considered normal.[10]

Doppler echocardiogram

Transthoracic echocardiography was performed using the Vivid 7 digital ultrasound system (GE Vingmed Ultrasound AS, Horten, Norway). Three cardiac cycles were recorded and digitally stored for further analysis. Left ventricle (LV) and right ventricle (RV) measurements were obtained according to the recommendations of the American Society of Echocardiography. LV ejection fraction (LVEF) was calculated using Simpson's biplane method.

Cardiac magnetic resonance imaging

Patients with HCM were submitted to CMR using a GE Signa 1.5-T system (Waukesha, Wisconsin). Cardiac images were obtained in the short and long axis in apnea and with pulse sequences synchronized with the electrocardiogram. To identify myocardial fibrosis using the late enhancement technique, 0.2 mmol/kg of gadolinium-based contrast was administered intravenously (Dotarem®, gadoteric acid - Gd-DOTA, Guerbet Aulnay-Sous-Bois – France).

Statistical analysis

Statistical analyses were performed using the SPSS software (Statistical Package for Social Science) for Windows, version 20 (SPSS Inc., Chicago, IL, USA).

In the descriptive analysis, the variables were expressed as absolute (N) and relative (%) frequencies and mean and standard deviation for continuous variables.

The Kolmogorov-Smirnov test was performed to evaluate the normal distribution of data. The Mann-Whitney test was used to compare the possible differences between the HCM groups, and the Student's t-test for independent sample was performed to compare the means of the groups.

A level of significance of 5% (p value < 0.05) was considered in the statistical analysis.

Results

The study included 100 subjects divided into two groups, 50 HCM patients and 50 healthy control subjects. The groups were similar regarding age and

gender (Table 1). The values found for serum Gal-3 measurements were low and showed no statistical differences between the two groups, HCM versus control, 10.3 ± 3.1 and 11.3 ± 2.6, p = 0.12 (Figure 1).

The patients with HCM were 72% males, with a mean age of 44 ± 12 years. All of them had preserved LVEF, mean septal thickness of 21.7 ± 5.2 mm and 29% had left ventricular outflow gradient > 30 mmHg at rest. Myocardial fibrosis was identified in 84% of these patients, and Gal-3 levels did not show any differences between the groups with present and absent myocardial fibrosis, 10.3 ± 3.4 and 10.1 ± 2.1, p = 0.59 (Table 2).

Table 1 - Comparison between the hypertrophic cardiomyopathy and controls groups

	HCM (n = 50)	Controls (n = 50)	p
Age, years, mean ± SD	43 ± 14	39 ± 10	0.09
Gender [M/F], n	36/14	35/15	0.41
Gal-3, mean ± SDP, ng/dL	10.3 ± 3.1	11.3 ± 2.6	0.42
Minimum - maximum	6.3 - 17.6	5.2 - 15.9	
Percentile			
25	8.7	9.3	
50	10.4	11.4	
75	12.8	13.2	

HCM: hypertrophic cardiomyopathy.

Discussion

In the present study, serum Gal-3 levels were low and did not show any differences between patients with HCM and the control group. Myocardial fibrosis was a common finding, present in 84% of HCM patients, but Gal-3 values also showed no differences between patients with and without fibrosis. Therefore, our findings suggest that other non-inflammatory mechanisms of myocardial structural remodeling are involved in the pathophysiology of fibrosis in this cardiopathy.

Galectin-3 is expressed by activated monocytes/macrophages and is involved in the regulation of inflammatory processes and pro-fibrotic pathways, acting as myocardial fibrosis mediator under conditions where necrosis and/or cellular apoptosis and consequent inflammatory reactions occur, described as a pathway of reparative fibrosis.[12,16] However, another collagen

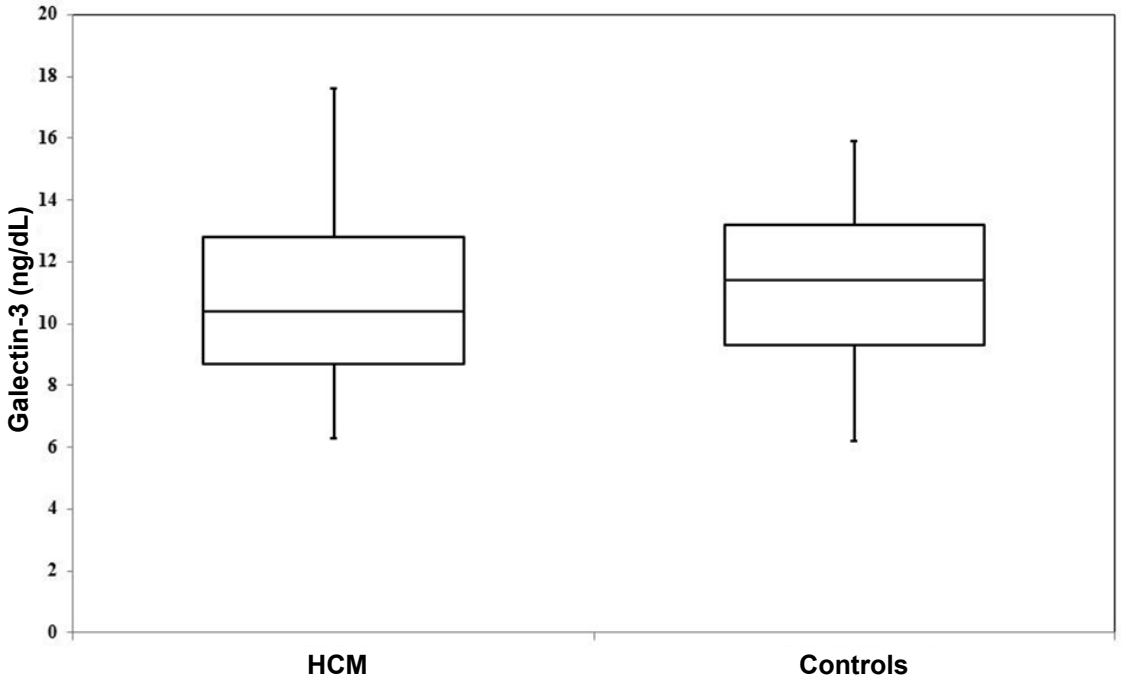

Figure 1 - Distribution of serum Gal-3 levels in patients with HCM and control subjects. The chart shows the medians (horizontal bars), the 25th and 75th percentiles (lower and upper limits of the boxes) and the lower and upper absolute values (error bars)

Table 2 - Comparison between patients with hypertrophic cardiomyopathy with and without myocardial fibrosis

	With fibrosis (n = 42)	Without fibrosis (n = 8)	p
Age, years, mean ± SD	44 ± 14	42 ± 14	0.85
Gender [M/F], n	33/9	3/5	0.65
Echocardiogram - mm, mean ± sd			
Septal thickness	21.7 ± 5.4	21.6 ± 4.3	0.91
Posterior wall thickness	11.7 ± 3.0	11.6 ± 2.1	0.94
Left atrial diameter	43. 0 ± 6.5	44.1 ± 6.9	0.70
LV diastolic diameter	43.5 ± 6.1	44.2 ± 2.9	0.82
Ejection fraction,%	66.5 ± 9.0	66.2 ± 3.7	0.94
Gal-3, mean ± SD, ng/dL	10.3 ± 3.4	10.1 ± 2.1	0.59
Minimum - maximum	6.3 - 17.6	8.0 - 12.8	

synthesis mechanism is known: the reactive pathway, in which fibrosis formation would be secondary to the neurohumoral activation without cardiomyocyte necrosis, with the following cellular mediators: angiotensin II, aldosterone and endothelin-1.[17-19]

In patients with HCM, cardiac fibrosis is associated with adverse left ventricular remodeling, arrhythmias and worse prognosis. Currently, it is not known what factors decisively contribute to accelerate collagen deposition between the hypertrophied myocardial fibers in patients with HCM, and different causal mutations are thought to play an important role, as well as other genetic polymorphisms and environmental factors.[20-22]

Kim et al.,[23] demonstrated in an experimental study that myocardial fibrosis occurs independently of cell damage and inflammation in this heart disease. When analyzing the hearts of transgenic mice with a cardiac beta-myosin mutation, in which hypertrophy had not yet developed, they reported the early activation of potent fibrosis regulatory pathways and collagen deposition, despite normal cardiac histological findings, demonstrating the existence of a pro-fibrotic environment even before the disease onset.[23] Thus, they corroborate our results, with low serum concentrations of Gal-3 and absence of an association with myocardial fibrosis.

The study by Hu et al.,[24] aimed to determine the prognosis of myocardial fibrosis associated with Gal-3 levels in patients with non-ischemic cardiomyopathy. The sample consisted of 105 patients with HCM, with an older mean age than that in our study, 52 ± 15 years, and they identified that the association of Gal-3 with fibrosis showed a more significant prognostic value. However, when evaluating serum levels of Gal-3 in patients with HCM with and without fibrosis, they also did not find any significant differences.[24]

The findings of the present study contribute to the knowledge of the mechanisms involved in myocardial fibrosis formation in HCM, guiding future lines of research aimed at studying the formation of fibrosis and ventricular remodeling and modifying the natural history of the disease.

Conclusion

In patients with HCM, the serum concentration of Gal-3 is low and is not associated with the presence of myocardial fibrosis, suggesting that the reparative pathway of fibrosis formation is little activated in this cardiopathy.

Limitations

Some limitations should be considered in the study, such as the relatively small number of patients, its cross-sectional design, which did not allow us to establish the prognostic value of the variables, and the evaluation of myocardial fibrosis, which was not quantitatively performed, making it impossible to calculate the correlation of myocardial fibrosis extension with Gal-3 concentrations.

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Author contributions

Conception and design of the research: Antunes MO, Arteaga-Fernández E. Acquisition of data: Antunes MO, Arteaga-Fernández E, Buck PC, Moreira CHV. Analysis and interpretation of the data: Antunes MO, Arteaga-Fernández E, Fernandes F, Soffiatti CD, Sabino EC. Obtaining financing: Antunes MO, Soffiatti CD. Writing of the manuscript: Antunes MO, Soffiatti CD, Buck PC. Critical revision of the manuscript for intellectual content: Antunes MO, Arteaga-Fernández E, Fernandes F, Mady C.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

There were no external funding sources for this study.

Study Association

This study is not associated with any thesis or dissertation work.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Faculdade de Medicina da Universidade de São Paulo under the protocol number 0665/9 . All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

References

1. Maron BJ. Hypertrophic cardiomyopathy: a systematic review. JAMA. 2002; 287(10): 1308-20.
2. Arteaga E, Ianni BM, Fernandes F, Mady C. Benign outcome in a long-term follow-up of patients with hypertrophic cardiomyopathy in Brazil. Am Heart J. 2005;149(6):1099-105.
3. Marsiglia JDC, Batitucci MCP, Paula F, Barbirato C, Arteaga E, Araújo AQ. Estudo de mutações causadoras de cardiomiopatia hipertrófica em um grupo de pacientes no Espírito Santo, Brasil. Arq Bras Cardiol. 2010; 94(1):10-7.
4. Maron BJ. Hypertrophic cardiomyopathy: a systematic review. JAMA. 2002; 287(10):1308-20.
5. Shirani J, Pick R, Roberts WC, Maron BJ. Morphology and significance of the left ventricular collagen network in young patients with hypertrophic cardiomyopathy and sudden death. J Am Coll Cardiol. 2000; 35(1): 36-44.
6. Arteaga E, Araújo AQ, Bernstein M, Ramires Felix JA, Ianni BM, Fernandes F, et al. Valor prognóstico da fração de volume de colágeno na cardiomiopatia hipertrófica. Arq Bras Cardiol 2009;92(3): 222-6.
7. Chan RH,Maron BJ,Olivotto I, etal.Prognostic value of quantitative contrast-enhanced cardiovascular magnetic resonance for the evaluation of sudden death risk in patients with hypertrophic cardiomyopathy. Circulation.2014; 130(6):484-95.
8. Maron MS, Maron BJ. Clinical impact of contemporary cardiovascular magnetic resonance imaging in hypertrophic cardiomyopathy. Circulation 2015;132(4):292-8.
9. Rowin EJ, Maron MS. The role of cardiac MRI in the diagnosis and risk stratification of hypertrophic cardiomyopathy. Arrhythmia & Electrophysiology Review 2016;5(3):197–202.
10. Ho JE, Liu C, Lyass A, Courchesne P, Pencina MJ, Vasan RS, et al. Galectin-3, a marker of cardiac fibrosis, predicts incident heart failure in the community. J Am Coll Cardiol 2012; 60(14):1249-56.
11. Madrigal-Matute J, Lindholt JS, Fernandez-Garcia CE, Benito-Martin A, Burillo E, Zalba G, et al. Galectin-3, a biomarker linking oxidative stress and inflammation with the clinical outcomes of patients with atherothrombosis. J Am Heart Assoc. 2014; 3(4):pii:e000785.
12. Sharma UC, Pokharel S, Van Brakel TJ, Van Berlo JH, Cleutjens JP, Schroen B, et al. Galectin-3 marks activated macrophages in failure-prone hypertrophied hearts and contributes to cardiac dysfunction. Circulation. 2004;110(19):3121-8.
13. De Boer RA, Daniels LB, Maisel AS, Januzzi JL Jr. State of the art: newer biomarkers in heart failure. Eur J Heart Fail. 2015;17(6):559-69.
14. De Boer RA, Yu L and van Veldhuisen DJ. Galectin-3 in cardiac remodeling and heart failure. Curr Heart Fail Rep. 2010;7(1):1-8.
15. Ho JE, Liu C, Lyass A, Courchesne P, Pencina MJ, Vasan RS, et al. Galectin-3, a marker of cardiac fibrosis, predicts incident heart failure in the community. J Am Coll Cardiol. 2012;60(14):1249-56.
16. Frangogiannis NG. Regulation of the inflammatory response in cardiac repair. Circ Res. 2012; 110(1):159-73.
17. Filipe MD, Meijers WC, Rogier van der Velde A, de Boer RA . Galectin-3 and heart failure: prognosis, prediction & clinical utility. Clin Chim Acta. 2015 Mar 30;443:48-5.
18. Sutton MG, Sharpe N. Left ventricular remodeling after myocardial infarction: pathophysiology and therapy. Circulation. 2000;101(25):2981-8.
19. Bohl S, Wassmuth R, Abdel-Aty H, Rudolph A, Messroghli D, Dietz R, et al. Delayed enhancement cardiac magnetic resonance imaging reveals typical patterns of myocardial injury in patients with various forms of nonischemic heart disease. Int J Cardiovasc Imaging. 2008;24(6):597-607.
20. Moon JC, McKenna WJ, McCrohon JA, Elliott PM, Smith GC, Pennell DJ. Toward clinical risk assessment in hypertrophic cardiomyopathy with gadolinium cardiovascular magnetic resonance. J Am Coll Cardiol 2003;41(9):1561-7.
21. Lombardi R, Betocchi S, Losi MA, Tocchetti CG, Aversa M, Marianna M, et al. Myocardial Collagen Turnover in Hypertrophic Cardiomyopathy. Circulation. 2003;108(12):1455-60.

22. Ho CY, López B, Coelho-Filho OR, Lakdawala NK, Cirino AL, Jarolim P, et al. Myocardial fibrosis as an early manifestation of hypertrophic cardiomyopathy. N Engl J Med 2010;363(6):552-63.

23. Kim JB, Porreca GJ, Song L, Greenway SC, Gorham JM, Church GM, et al. Polony multiplex analysis of gene expression (PMAGE) in mouse hypertrophic cardiomyopathy. Science 2007;316(5830):1481-4.

24. Hu DJ, Xu J, Du W, Zhang JX, Zhong M, Zhou YN. Cardiac magnetic resonance and galectin-3 level as predictors of prognostic outcomes for non-ischemic cardiomyopathy patients. Int J Cardiovasc Imaging. 2016 Dec;32(12):1725-1733.