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Original Contributions

Morphological and histopathological evaluation of autopsied patients with hypertensive cardiopathy

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ABSTRACT

Background: Physiopathological processes in hypertensive heart disease are controlled by complex interactions between cardiomyocytes, extracellular matrix, microvasculature and other cells present in the myocardium.
Objective: To analyze morphological changes in hypertensive cardiopathy and to describe and compare findings in order to help clarify determinant factors.
Methods: 42 fragments of the left ventricular myocardium and circumflex branch of the left coronary artery were obtained from individuals autopsied at the Clinical Hospital of the Federal University of Triângulo Mineiro (UFTM) in the period ranging from 1984 to 2018. Groups were split into individuals with hypertensive heart disease (HD) and individuals without heart disease (ND). Wall thickness was measured with a digital caliper and Computed Tomography. Quantification of collagen fibers was conducted by computerized morphometry and mast cell density was assessed by immunohistochemical methods.
Results: There was a significant increase of heart weight in the HD group compared to the ND group, ($p = 0.0002$). There was a significant increase of thickness of the middle third of the free wall in the HD group compared to the ND group, ($p = 0.04$). There was a significant increase of collagen fibers in the left ventricle in the HD group compared to the ND group, ($p < 0.0001$). Concerning mast cell density, there was a significant increase in the left ventricle of individuals with HD immuno-labeled by the set anti-chymase/anti-tryptase ($p < 0.0001$). There was a significant increase of mast cell density in the circumflex branch of the left coronary artery of individuals with HD immuno-labeled by the set anti-chymase/anti-tryptase ($p = 0.01$).
Conclusions: Mast cells are involved in the development of hypertensive heart disease, contributing to the remodeling of collagen fibers in this disease.

1. Introduction

Systemic arterial hypertension (SAH) affects about 1 billion people globally, remains the main factor of morbidity and mortality and is predicted to be the main risk factor for cardiovascular diseases by 2040 [1,2]. SAH is an independent risk factor for many clinical conditions such as myocardial infarction, chronic kidney disease, ischemia, hemorrhagic stroke, heart failure and premature death [3]. Essential or idiopathic hypertension is responsible for about 95% of cases and corresponds to a multifactorial disease, in which genetic predisposition and environmental factors interact for development of the disease [4].

One of the consequences of untreated SAH is the so-called hypertensive cardiopathy that induces ventricular hypertrophy. Physiopathological processes in hypertensive heart disease are controlled by complex interactions between cardiomyocytes, extracellular matrix, microvasculature and other cells present in the myocardium [5]. Hypertensive heart disease is characterized by changes in myocardial structure (growth, apoptosis, neoformation of interstitial and

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perivascular collagen fibers) that induce myocardial remodeling, leading to left ventricular dysfunction and hypertrophy, which may induce cardiac failure [6]. From initial changes in cardiac remodeling, there is a progressive accumulation of collagen fibers (fibrosis), also causing abnormalities in intramyocardial vasculature (perivascular fibrosis) [7], triggering an unbalance in the energetic supply for cardiomyocytes [8].

Studies in *postmortem* specimens of human hearts have shown increased collagen in patients with hypertensive heart disease compared to normotensive patients, but other morphological findings involving hypertensive heart disease have not been very well documented in the literature [9-11]. This is due, in part, to clinical disinterest and also, many of these evaluations occur through echocardiographic analyzes, with emphasis on cardiac mass and volume studies. Despite a high demand for minimally invasive autopsies, few studies have investigated the real accuracy of findings in diagnosis of the cause of death [12]. Therefore, the purpose of our study in material from necropsies is to analyze morphological changes in hypertensive cardiopathy and to describe and compare findings in order to help clarify determinant factors.

2. Material and methods

This is a quantitative, human, observational, cross-sectional, retrospective and descriptive study. We evaluated 42 hearts from individuals autopsied in the Clinical Hospital of Federal University of Triangulo Mineiro (HC/UFTM), Uberaba-MG, from 1984 to 2018. Autopsy protocols were analyzed to obtain data such as age, gender and race. Individuals with 18 years of age or older were selected and divided into two groups: (1) individuals with hypertensive heart disease (HD) (n = 21) and (2) individuals without heart disease (ND) (n = 21). We excluded from this study cases with incomplete autopsy reports; possible causes of secondary hypertension as renal disease, tumors, vascular or endocrine disorders and cases with Chagas' disease or any type of cardiopathy.

The following macroscopic characteristics were assessed: atherosclerosis in aortic root; presence of atherosclerosis in the circumflex branch of left coronary artery; thickness of the distal third of left ventricle free wall; cardiac aspect; epicardial fat; fibrous thickening of mitral, aortic, tricuspid, and pulmonary valves as well as the thickness of interventricular septum and right ventricle.

For analysis of wall thickness, hearts were sectioned carefully in a cross section, exposing the left ventricle. The middle third of the free wall was selected and used for measurements [11]: with a digital caliper (100.174B) (Digimess Precision Tools Ltda.) and by using the Computed Tomography (CT) equipment Toshiba Aquilion 64 (TX-101 A/H) (Toshiba America Medical Systems Inc.). The *software* for image analysis was eFilm LITE 2.1.0 (Merge Healthcare), which used 1-mm-thick slices with 0.5 mm interpolation of image reconstruction, which increases the quality of image reconstruction processed in other planes and even in three dimensions, available at HC-UFTM Radiology Service; thicknesses of the interventricular septum and right ventricle were also measured. Trabeculations of endocardium, epicardial fat and papillary muscle were excluded from measurements.

The intensity of the processes was classified semi quantitatively according to the predominance in the structure analyzed as follows: absent; mild, in case of involvement of up to 25%; moderate, in the case of involvement of 26 to 50% and severe in the case of involvement of > 51% [13].

The following microscopic characteristics were evaluated: percentage of collagen fibers of the middle third of the left ventricular free wall, percentage of collagen fibers in circumflex branch of the left coronary artery, mast cell density of the middle third of the left ventricular free wall, mast cell density in the circumflex branch of the left coronary artery. The number of fields for evaluation and quantification of collagen fibers of left ventricle was defined by the test of cumulative means [14]. We divided the histological sections into four quadrants and digitalized forty fields of each fragment. The area of collagen fibers under polarized light showed a birefringent aspect, ranging from greenish yellow to reddish Orange. Collagen fibers were marked by the observer to obtain the percentage of collagen per field. Thus, a camera attached to the microscope scanned the quantized image field. The morphometric analysis was performed using the image analyzer LeicaQWin® Plus (Leica Microsystems).

Quantification of immunostained mast cells was performed using a video camera connected to a conventional light microscope and to a computer with AxioVision® Rel 4.9.1 (Carl Zeiss) image analysis software. Mast cells were quantified along the slide using final magnification of 500× in a microscope field of 0.1520mm^2. Slides were divided into four quadrants and, in each quadrant, ten randomly selected fields were quantified. The density of mast cells of each slide was determined by dividing the total number of mast cells in quantified fields by the total area of the field, expressed in mast cells/mm^2.

Data analysis was performed using the software GraphPad Prism® 7 (GraphPad Software). In order to check the distribution of variables, Shapiro-Wilk statistical test was applied. When distribution was normal, Student *t*-test (t) was used to compare groups. When the distribution was not normal, Mann-Whitney test (U) was used. The correlation between two variables with normal distribution was analyzed using Pearson's test (r), and for those presenting non-normal variance, Spearman test (rS) was used. Differences were considered statistically significant when p was lower than 5% ($p < 0.05$). For semi-quantitative data expressed in different degrees (mild, moderate or severe), the frequency test X^2 (chi-square) and the Fisher's exact test were used.

The Research Ethics Committee of UFTM through the protocol CAAE n ° 61,526,216.5.0000.5154 approved this study.

3. Results

(See Tables 1–3.)

4. Discussion

In our study, there was a significant increase in cardiac weight in the HC group compared to NC group. Literature evidences the influence of anthropometric data and body mass index on heart mass, especially

Table 1
General characteristics of the sample of 42 individuals with (HD) and without cardiopathy (ND), autopsied in the period from 1984 to 2018.

Variables	With cardiopathy (n = 21)	Without cardiopathy (n = 21)
Mean age (min-max)	50.8 (26–81)	54.2 (30–80)
Gender n (%)		
Male	14 (66.67%)	14 (66.67%)
Female	7 (33.33%)	7 (33.33%)
Skin color n (%)		
White	12 (57.14%)	13 (61.90%)
Non White	9 (42.86%)	8 (38.10%)
Atherosclerosis (aortic root) n (%)		
Without	1 (4.76%)	2 (9.52%)
Mild	10 (47.62%)	11 (52.38%)
Moderate	3 (14.29%)	3 (14.29%)
Severe	7 (33.33%)	5 (23.81%)
Atherosclerosis (left coronary) n (%)		
Without	4 (19.04%)	7 (33.33%)
Mild	9 (42.86%)	10 (47.62%)
Moderate	5 (23.81%)	3 (14.29%)
Severe	3 (14.29%)	1 (4.76%)

n: sample; min: minimum; max: maximum.
Aortic root: $X^2 = 0.714$; $p = 0.869$.
Left coronary: $X^2 = 2.371$; $p = 0.499$.

Table 2
Morphometric analysis of the middle third of the left ventricular free wall and the circumflex branch of left coronary artery in hearts of 42 autopsied individuals with (HD) and without cardiopathy (ND) from 1984 to 2018.

Groups	HW (g)	TLVDC (cm)	TLVCT (cm)	CFLV (%)	CFLC (%)	MCDLV (cells/mm^2)	MCDLC (cells/mm^2)
	mean ± standard error or median (minimum-maximum)						
With cardiopathy	414.6 ± 24.68	1.144 ± 0.58	1.25 (1–1.6)	1.165(0–91.42)	4.57 (0–40.08)	4.10 ± 0.47	2.14 (0.82–9.21)
Without cardiopathy	304.3 ± 11.35	0.99 ± 0.04	1.3 (0.5–1.3)	0.80(0–20.11)	3.62 (0.04–84.42)	1.88 ± 0.2	0.99 (0–2.63)
	t = 4.06, p = 0.0002*	t = 2.08; p = 0.04*	U = 12.5; p = 0.44	U = 311,775; p < 0.0001*	U = 109,884; p = 0.22	t = 4.36; p < 0.0001*	U = 36.5; p = 0.01*

HW: heart weight; TLVDC: thickness of the middle third of the free wall of the left ventricle by the digital caliper; TLVCT: thickness of the middle third of the free wall of left ventricle by computed tomography; CFLV: percentage of collagen fibers from the middle third of the free wall of left ventricle; CFLC: percentage of collagen fibers from the left coronary artery circumflex branch; MCDLV: mast cell density of the middle third of the left ventricular free wall; MCDLC: mast cell density of the left coronary artery.

in evaluations of heart weight. Several authors consider the body mass index (weight and height) in interpretation of their results [15,16]. Researchers emphasize that every cardiac mass evaluation should be adjusted to reference data obtain from necropsies performed in the population to be studied. They vary with age, sex, ethnicity, size and body composition [17,18]. Some studies have demonstrated the relationship of excess weight with left ventricular hypertrophy and concentric and eccentric remodeling, and with diastolic dysfunction followed by long-term systolic dysfunction, indicating a direct effect of body composition on cardiovascular system. [19,20]. Regarding left ventricular thickness, there was a significant difference between the HC group and NC group. Despite the significant difference, literature considers left ventricle hypertrophy (LVH) when LV thickness is above 1.5 cm [21,22]. The mean thickness in our studies was 1.144 cm measured by digital caliper and 1.26 cm measured by CT. As it was a study of linear dimensions (thickness) of cardiac walls, it was decided not to assess ventricles' weight, due to the natural difficulty in separating interventricular septum between left and right myocardium, and due to fragments previously removed from hearts for other studies.

Inside the heart, systemic arterial hypertension can be considered a factor responsible for structural and functional changes [23]. In our study, we found an increase in collagen fibers in the hypertensive heart disease group compared to the no heart disease group. These findings confirm the literature, which reports that among morphological changes of hypertensive heart disease, fibrosis is one of the changes found in most hypertensive patients, characterized by diffuse or excessive accumulation of collagen fibers, increasing myocardial stiffness, which can lead to left ventricular dysfunction and, finally, heart failure [24-26]. This reaction fibrosis is characteristic of pathological processes that trigger myocardial hypertrophy [27]. The mechanisms responsible for the progression of left ventricular hypertrophy include not only a response to mechanical stress, but also the influence of neuro-hormones, growth factors and cytokines [28].

It has also been demonstrated that the serum ratio of matrix metalloproteinase-1 (MMP-1) and its tissue inhibitors are altered in patients with hypertensive heart disease [29] and that patients with failure in ejection fraction due to left ventricular chamber dilatation presented changes in distribution of collagen fibers, mainly perivascular and scar, and considerable increase in MMP-1 [27].

In the present study, we found a significant increase in the density of mast cells in the left ventricle of individuals with hypertensive heart disease. Mast cells are the main mediators of myocardial remodeling, which occurs via activation of MMPs, mainly by the release of tryptase, which cleaves the pro-MMP-3 precursor, activating MMP-3 which then activates MMP-1 and the release of chymase, activating MMP-2 and MMP-9 [30-35]. This remodeling appears to be critical to the progression of left ventricular hypertrophy.

Experimental studies that explain our results demonstrated increased mast cell density in left ventricular overload conditions and myocardial remodeling. The products of mast cell degranulation, besides activating the innate immunity, are of pro-inflammatory, pro-fibrogenic and hypertrophic nature, and among these, are the transforming growth factor β (TGF-β), histamine, chymase, tryptase, interleukin-4 (IL-4) and tumor necrosis factor α (TNF-α), [36-40] thus correlating with the significant increase of collagen fibers in the left ventricle of patients with hypertensive heart disease.

5. Conclusions

It could be observed that, in patients with hypertensive heart disease, there is an increase in collagen fibers in the left ventricle. This reaction fibrosis is a fundamental process for triggering myocardial hypertrophy. The increase in the density of mast cells in the left ventricle and circumflex branch of left coronary artery of patients with hypertensive heart disease observed in the study allowed us to conclude that mast cells have an important role in myocardial remodeling in hypertensive heart disease. Additional studies will contribute to complete elucidation of the mechanisms of collagen turnover, so that therapeutic targets may be determined in order to regulate the actions of the main proteases released in the activation of mast cells.

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Table 3
Macroscopic changes in hearts of 42 autopsied individuals with (HD) and without cardiopathy (ND) from 1984 to 2018.

Macroscopic Changes	HD (n = 21) n(%)				ND (n = 21) n(%)				p
Fibrous thickening	Absent	Mild	Moderate	Severe	Absent	Mild	Moderate	Severe	
Mitral	1(4.76)	7(33.33)	8(38.1)	5(23.8)	–	8(38.09)	6(28.57)	7(33.33)	0.640
Aortic	7(33.33)	7(33.33)	3(14.28)	4(19.04)	7(33.33)	5(23.8)	3(14.28)	6(28.57)	0.865
Tricuspid	6(28.57)	8(38.09)	7(33.33)	1(4.76)	6(28.6)	9(42.85)	5(23.8)	1(4.76)	0.946
Pulmonar*	18(85.7)	3(14.28)	–	–	15(71)	5(23.8)	–	–	0.453
Epicardial fat	5(23.80)	1(4.76)	10(47.6)	5(23.80)	5(23.8)	7(33.33)	6(28.57)	3(14.28)	0.111

Chi-square tes (X^2); * Fisher test (F).

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