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ORIGINAL ARTICLE

The use of a high-power laser on swine mitral valve chordae tendineae

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Abstract Worldwide, rheumatic fever remains a significant cause of mitral valve insufficiency. It is responsible for approximately 90 % of early childhood valvular surgeries in Brazil. Elongated or flail chordae are frequently responsible and require surgical correction. The purpose of this study was to analyze and compare the histological tissues of the mitral valve chordae and the mechanical resistance generated by the chordae, both with and without the application of a high-power laser. Twenty normal porcine mitral valve chordae were measured and divided randomly into the following two groups: control group (not subjected to a high-power laser) and laser group (subjected to photonic irradiation). Laser surgery was performed under controlled conditions, using following parameters: $\lambda = 980$-nm wavelength, power = 3 W, and energy = 60 J. A mechanical test machine was used in combination with a subsequent histological study to measure chordae tensile properties. A histological analysis demonstrated a typical collagen bundle arrangement in the control group; however, under a particular reached temperature range (48), the collagen bundles assumed different arrangements in the laser group. Significant reductions in the chordae tendineae lengths and changes in their resistance in the laser group were observed, as these chordae exhibited less rigid fibers. The chordae tendineae of normal porcine valves subjected to a high-power laser exhibited its length reduction and less stiffness compared to the control group. A histological analysis of the laser treatment specimens demonstrated differences in collagen bundle spatial organization, following slight changes into tissue temperature.

Keywords Chordae tendineae · Mitral valve · Mitral valve repair · High-power laser · Rheumatic disease

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Introduction

Mitral insufficiency (MI) is difficult to treat due to the mitral valve complex structure. MI may occur due to changes in valvar structure secondary to either inflammatory or degenerative diseases, such as rheumatic fever, myxomatous degeneration, idiopathic valvular prolapse, or postischemic heart disease [1].

Rheumatic fever is responsible for over 30 % of the cardiac surgeries in Brazil. Previous studies have noted that these surgeries occur in 19 % of cases before the age of 4 [2]. Patients with rheumatic mitral insufficiency exhibit greater difficulty with plastic repair. However, these patients benefit from preservative surgery, when it is available. This technique is more complex and involves annuloplasty, string transposition, and shortening, as well as leaflet resection of the chordae tendineae [3]. Valve surgery still remains a therapeutical challenge to cardiac surgeons. Immediate operative mortality ranges from 2.6 to 4 % in patients with double mitral lesions [1]. Several studies have demonstrated that subsequent restenosis may occur following an open mitral commissurotomy. In patients with insufficiency or double mitral lesions, the presence of elongated chordae tendineae is not uncommon and requires surgical correction.

Several techniques have been used to treat these abnormalities, although no literature reports regarding specific chordae retraction techniques, such as applying a high-power laser (HPL) to shorten the chordae tendineae. Mitral valvuloplasty in the setting of rheumatic fever is quite controversial. It has been heavily discussed in the literature, due to the discordant results reported in various publications. Valve repair difficulty remains a therapeutical challenge and the confounding interference caused by new disease outbreaks [3, 4].

Laser surgery or simply HPL is used for tumor resection and lesion ablation, as well as for the delicate and precise cutting of target tissues, in the case of transmyocardial laser revascularization treatment (TLR, CO_2-800 W). Depending on the tissue absorption coefficient, a photothermal effect according to the temperature on biological target may result hyperthermia (below 45 °C), tissue welding (50–60 °C), coagulation (60–80 °C), protein denaturation (80–95 °C), carbonization (100 °C), vaporization (above 400 °C), and cutting (1.500 °C) [5]. There are several benefits to employing a laser in different surgeries, including the prevention and reduction of bleeding, swelling, pain, and infection risks. Furthermore, the HPL allows to a high degree of precision when surgical incisions is needed [5, 6].

The aim of this study was to compare the histological findings and mechanical strengths of the chordae tendineae of porcine mitral valves, both with and without surgical laser procedure. In order to evaluate this method as a treatment option for chordae tendineae elongation secondary to mitral valve insufficiency.

Materials and methods

Heart valve selection criteria

This study design was submitted to and approved by the Ethics and Research Committee of InCor/HC (Heart Institute of Clinics Hospital) at Medical School in University of São Paulo.

This was an experimental and descriptive study, in which four mitral valves from young (6–12 months of age), healthy swine were used. They were obtained from a slaughterhouse, under the supervision of the Inspection Department of the Federal Ministry of Agriculture and the Health Surveillance Department of the State Health Secretariat. At the relevant site, the valves were rinsed in saline solution prior to removal and were immediately preserved in a sterile glycerin liquid container at room temperature in accordance to previous study recommendations [7–9]. The material was subsequently transported to the laboratory of the Lasers and Applications Center (CLA) of the Energy Institute and Nuclear Research (IPEN-University of São Paulo). In the laboratory, the mitral valves were rehydrated in three different receptacles containing 0.9 % saline solution, at room air for 30 min each. Once rehydrated, 20 chordae tendineae were divided randomly into the following two groups:

Control group (G1): No laser was used ($n = 10$)
Laser group (G2): Laser procedure onto the chordae tendineae ($n = 10$)

Laser treatment

Additional treatment doses were tested before the present study. It has been tested emitting 780-, 980-, and 1064-nm wavelengths (λ), powers (P) of 2.0 and 3.0 W, and times (t) of 15 and 30 s. Less thermal damage associated with the desired effect (chordae shortening) was observed, within the following parameters: in G1, no laser was utilized and in G2, the chordae tendineae were treated using a CW Diode Laser (MediLaser model-DMC, São Carlos, SP, Brazil). The semiconductor laser has a continuous operation mode and a 400 μm fiber emitting 980-nm wavelength (the ideal wavelength), power of 3.0 W, time of 20 s, irradiance of 1.88 W/cm^2, energy (E) of 60 J, fluence of 3.76 J/cm^2, spot size of 0.0016 cm^2, and diameter of 0.0451 cm, respectively. The laser was irradiated perpendicularly to the chordae tendineae, along the chordae central portions at 1-mm distance, using a servo motor device (ESP 300 model, Newport, USA).

The power was measured by Power Meter equipment (FieldMaster GS, Coherent Instruments, USA) during each use. A single operator performed the procedure. The mitral valve and its chordae tendineae were positioned on Styrofoam and fixed with metal pins on workbench flat surface. The mitral valve was hydrated with 0.9 % saline solution as needed.

Computerized measurements

ImageJ software, version 1.6, was employing to measure the chordae tendineae length. A motion control system (Newport, Irvine) was applied to rotate the sample in both a constant and repeatable manner.

Thermography

A thermography camera (ThermaCam FLIR SC3000 Systems, USA) was used throughout the HPL procedure to analyze the chordae tendineae temperature. Using this equipment, infrared images were recorded at 60-Hz rate. The temperature in the laboratory remained constant at 22 °C throughout all experiments, and the chordae emissivity was 0.98. The images were acquired and analyzed by ThermaCAM Research software (FLIR System, USA).

For image acquisition, the camera was positioned 0.1 m away from the samples to focus on the chordae tendineae. The laser irradiation was perpendicular to the chordae, as the camera was positioned lateral to the sample, so that the laser beam temperature would not interfere with the sample temperature measurements.

The temperatures measured by the thermography camera were surface temperatures. As the samples were thin, the laser was able to reach the target tissue posterior surface and produce different temperatures throughout the sample depth.

Sample preparation

Seven samples were preserved in 10 % formalin for optical microscopy analysis (hematoxylin-eosin (HE), Masson's trichrome, and Picrosirius red) and six samples were preserved in glutaraldehyde for transmission electron microscopy (TEM). The formalin-preserved samples were submitted to conventional histological processing and staining at the Pathology Department of Heart Institute from University of São Paulo, School of Medicine (InCor HC FMUSP). Processing for TEM was carried out at the Cellular Biology Laboratory from University of São Paulo, School of Medicine. The remaining seven samples were preserved in 0.9 % saline solution and immediately sent to the Biomedical Sciences Institute (ICB-USP) for mechanical analysis.

Mechanical evaluation

A mechanical tensile device (EMIC line DL-200 MF) was employed to evaluate the mechanical properties of the chordae tendineae. The tensile test model was used to determine the maximum force (Fmax), displacement (Desl max), and stiffness [10]. The chordae tendineae has a mean length of 12 mm, and the central region (4 mm) was always irradiated. This target area underwent a mechanical testing. During each test, postlaser irradiation or not, the first one third of the sample was attached to the top of the machine; the final one third was attached to the bottom of the device, leaving exactly 4 mm available for mechanical testing. Tensile strength was applied to the target tissue until it ruptured. A tensile speed of 5 cm/min up was used to rupture the tissue, at which time the test was stopped.

Correction for chord diameter was performed for each calculated force (strength divided by diameter) to determine both the maximum rupture force and the degree of material displacement (elongation). The angle formed by the curves was described with respect to whether the material was either more or less rigid (α angle). For this evaluation, a line atop each curve was drawn and the observed angle was analyzed.

Statistical analysis

Initially, all variables were descriptively analyzed. To compare the means of the two groups, Student's *t* test was used. To test the homogeneity between the proportions (as the expected frequencies were sometimes lower than 5), the Fisher's exact test was utilized. The Wilcoxon nonparametric test was employed to compare both the preperiod and the postperiod in each group. The significance level for all tests was set at 5 %.

Results

The prelaser chordae tendineae lengths and diameters were similar in both groups; in G1, they were 12.93 and 0.88, respectively, and 15.04 and 0.76, respectively, in G2. There was a significant decrease noted in the chordae tendineae following laser irradiation, when compared with the initial lengths ($p = 0.005$). However, the chordae diameters did not change significantly with procedure (0.345).

Thermography

The chordae tendineae area was selected, and the maximum temperatures of this region were recorded during the laser irradiation procedure. Any measurements taken during the first 10 s and following 30 s of treatment were disregarded. Therefore, only a total of 20 s of laser irradiation was studied. The maximum temperature reached was 48 °C (Fig. 1).

Histologic and electron-microscopic analysis

The preserved valvular structures and chordae were identified using an optical microscope. The tissue showed a homogenous bluish color when subjected to Masson's trichrome staining and exhibited normal collagen birefringence, when stained with Picrosirius red (Fig. 2a–c). However, staining changes were observed in the laser group (G2), in which the chordae exhibited hyaline areas occupying variable extensions of its thickness. Additionally, dark red areas were observed in the central region of chordae in Masson's trichrome stained slides, besides a lower birefringence of collagen in the Picrosirius red stained sections (Fig. 2d–f).

Using a transmission electron microscope, the control chordae (G1) showed dense, cross-sectioned collagen bundles

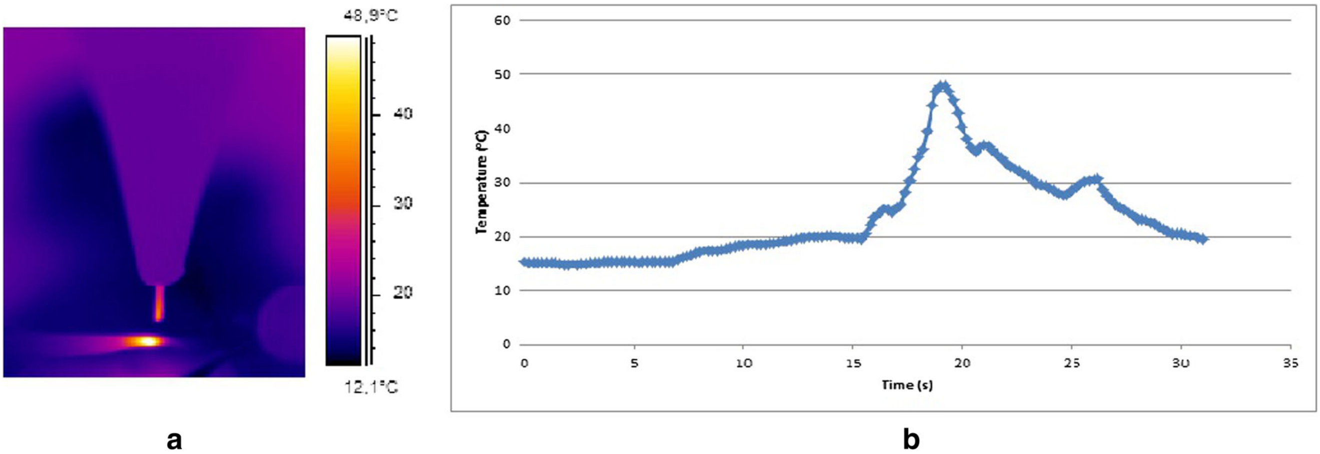

Fig. 1 Thermal image of chordae tendineae during laser irradiation (a). Time-temperature curve shows the maximum temperature reached in the chordae tendineae (48 °C) (b)

as expected for normal tissue (Fig. 3). The collagen fibers in the laser group (G2) were randomly grouped and rearranged in different directions. Moreover, the collagen bundles in the laser group chordae exhibited smaller diameters compared with the control ones (Fig. 4).

Mechanical study

Figure 5 depicts the mechanical tensile test, up to the chordae tendineae rupture point. The *Y*-axis represents the strength in N and allows for the material determination with the greatest strength during the stretch test. The top spot represents the maximum strength of each material at the time of rupture. The *X*-axis represents the degree of material displacement (mm) during the tensile test.

The G2 exhibited a lower breaking strength than G1. G2 showed higher displacement values than G1; additionally, G2 exhibited significantly greater elongation ($p = 0.05$). Moreover, G2 exhibited lower stiffness.

Discussion

Elongated chordae tendineae requiring surgical correction are a frequent finding in the setting of mitral insufficiency in children with rheumatic heart disease. In the literature, numerous

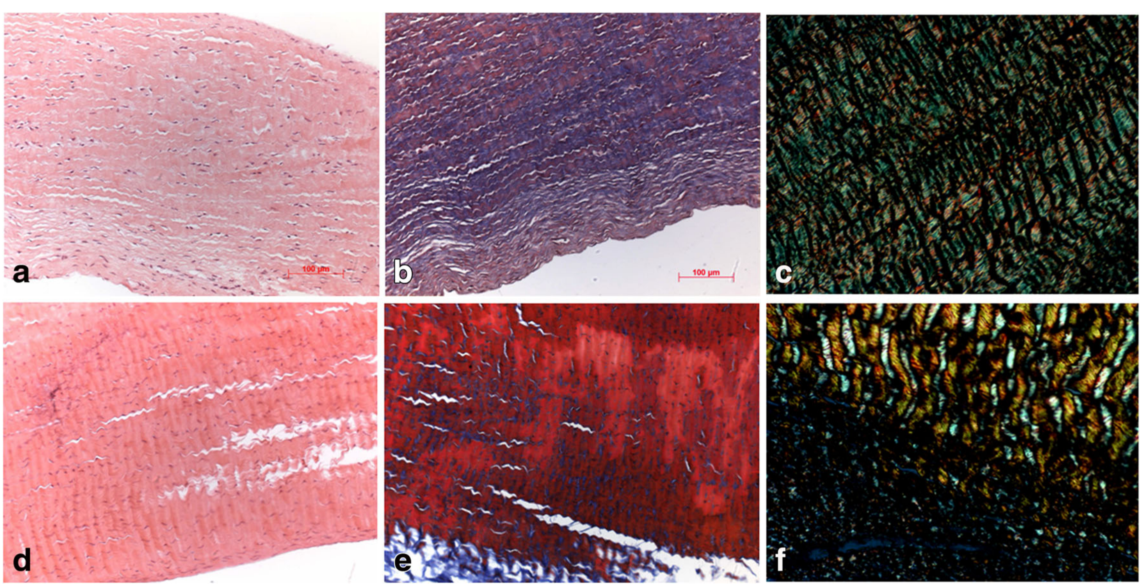

Fig. 2 Photomicrographs of the control chordae (a–c) and the ones submitted to laser (d–f). Compare the wavy and regular collagen bundles in the normal chord stained by hematoxylin-eosin (a) to the hypereosinophilic and partially disrupted fibers in the one from the laser group (d). Masson's trichrome stain showns the typical bluish color of the collagen in the control (b) as compared to the homogeneous red color in the chord from the laser group (e). Sirius red staining observed under polarized light shows in the control chord the diffuse and regular polarization of the collagen fibers (c) whereas in the laser group, there were focal areas with low or lacking polarization (lower half of f). Original magnification of the objective = ×20 for all photomicrographs

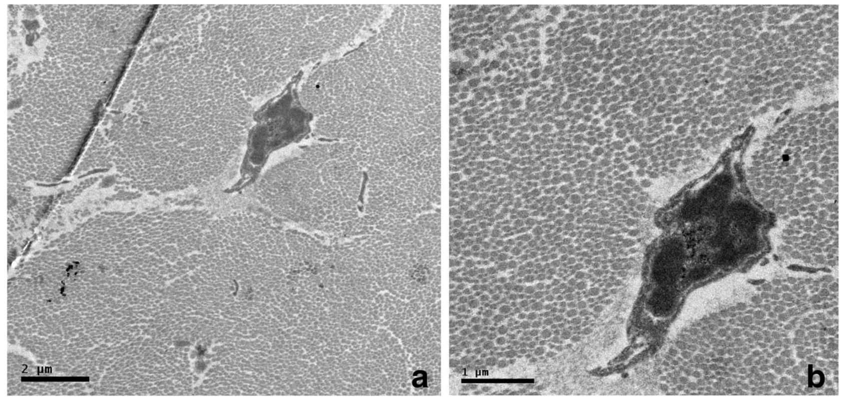

Fig. 3 Electronic micrographs depicting the ultrastructure of normal valvular chordae (G1). Dense and cross-sectioned collagen bundles are noticeable. Original increase ×10,000 (**a**) and ×25,000 (**b**)

surgical techniques have been proposed, although not all of these techniques have proven to be successful. Advances in surgical techniques are warranted to decrease immediate mortality rates following corrective surgery, preventing the need for additional operations. In the last half century, the introduction of the HPL in medicine, particularly in the fields of ophthalmology and dermatology, has resulted in unconceivable progress. The surgical diode laser, which has been commercially available since the 1990s, has been used increasingly in several medical procedures [11].

Structural tissue changes may occur due to photothermal effects after laser surgery. The lesion process may be caused by an imbalance in the collagen type I and III proportions. Either the levels of collagen I and III into the chordae tendineae can be altered or the target fibers may undergo changes in their spatial orientation. Each of these factors causes a significant influence on the tissue and induces changes in its mechanical properties, reducing strength and altering the stiffness [10].

In ophthalmology, thermally mediated collagen shrinkage has been reported to treat retinal detachment and create a selective collagen welding in the posterior inner lining membrane; this procedure does not affect the corneal epithelium and crystalline lenses [12]. In orthopedics, in vitro studies have demonstrated that lasers decrease the joint laxity associated with ligament reconstructions [13]. Furthermore, cardiac laser treatment can be used for thermally mediated scar shrinkage, which averts infarct expansion following myocardial infarction [14].

The histological analysis demonstrated that chordae tendineae subjected to laser procedure exhibited changes in the usual staining features observed at hematoxylin-eosin (HE), Masson's trichrome, and Picrosirius red techniques. Hyaline is a common finding in target tissues subjected to temperature changes, as demonstrated in the surgical tissue specimens subjected to electrocautery. However, the red Masson staining observed in this study has been only rarely reported. Chvapil et al., who described that the characteristic blue-green color of native collagen bundles changed to red, when collagen was subjected to heat, described it several years ago in the study [15]. According to Whittaker et al., collagen birefringence decreases above 70 °C; temperatures above 80 °C are associated with changes in fiber organization from wavy to straight following radiofrequency exposure, which was demonstrated both in vitro and in vivo, using dog hearts [16]. They investigated that the lesions created at lower temperatures would not expand, as undamaged collagen will resist stretching. Therefore, the Picrosirius red stained collagen visualized under polarized light demonstrated histologic temperature signatures corresponding to collagen denaturation.

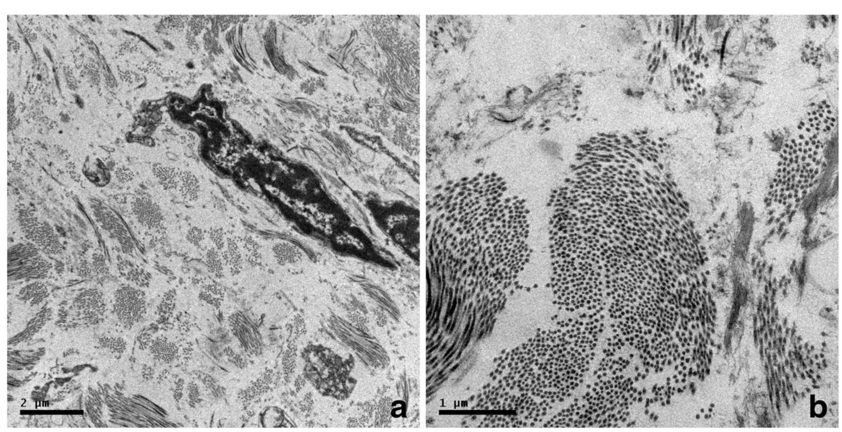

Fig. 4 Electronic micrographs depicting the ultrastructure of valvular chordae subjected to an HPL. Collagen bundles that separated and dispersed in several directions are noticeable. Apparently, these bundles are smaller in diameter than those in G1. Original increase ×10,000 (**a**) and ×25,000 (**b**)

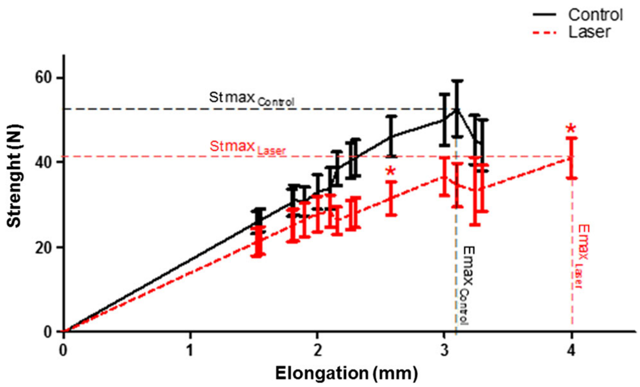

Fig. 5 G2 and G1 chordae tendineae mechanical properties up to tissue rupture. Fmax stands for maximum strength and Desl max, for maximum displacement. *Statistically significant differences ($p = 0.05$)

In our histological study, similar collagen changes were observed.

Iglésias et al. studied hearts with endomyocardial fibrosis and described a collagenized tissue band featuring similar tinctorial changes. Nevertheless, this group did not provide an explanation for the phenomenon in the setting of human disease [17]. Such findings can correspond to a global marker of tissue injury, as well as a marker of collagen fiber space restructuring in the chordae restricted to this experimental model. However, one of our study limitations was that there was no vital reaction since the tissues were subjected to the laser *in excised valvar tissue*. Therefore, additional in vivo studies are required to determine the healing pattern of laser-induced tissue injury in cardiac valves.

Furlanetto et al. used an argon laser (power from 0.5 to 2.5 W, from 2 to 105 s) to treat the mitral chordae tendineae of corpses within 12 h of death. They observed a shortening in the chordae and a tensile strength that varied slightly, although not significantly, compared with the strings not subjected to laser irradiation. In their study, a histological analysis demonstrated connective tissue coagulation necrosis in the chordae [18]. However, that study lacked information regarding the methodology used, compromising the quality of their work and their reproducibility.

In our study, a 980-nm wavelength (ideal wavelength), with 3-W power and 20-s time, was used to treat the mitral valve chordae tendineae. The tissue temperatures were evaluated throughout the experiment using step-by-step thermography; the target tissue was maintained at a lower temperature (<48 °C) throughout the experiment. There was a statistically significant difference noted with respect to chordae shortening and elongation between G2 and G1.

Additionally, changes in the chordae mechanical properties were observed post-HPL irradiation. The maximum strength required for the chordae tendineae to rupture in G2 was lower than in G1; G2 also exhibited greater displacement than G1. G2 exhibited greater tissue elongation (Fig. 5), which was indicative in the stiffness reduction of the target tissue. In this study, only one model of maximum force rupture was utilized. Therefore, it was not possible to comment on chordae elasticity, as the elasticity evaluation requires cyclic tests. Caution is warranted regarding the data presented here, as it pertains to the laser application to normal chordae tendineae. It is unknown whether this mechanical behavior may be generalized to the loose, elongated chordae that appear in the mitral insufficiency setting, as normal porcine valves do not undergo to these pathological changes.

Few studies have examined chordae tendineae resistance following HPL treatment; Jensen et al. quantified the maximum stretch chordae can endure [19]. The system that these authors developed allows the precise quantification and direction of the forces exerted on the posterior left ventricular wall during systole. Lobo et al. determined that the primary chordae tendineae resistance in the mitral valve in humans is related to its thickness and elongation at the moment of tensile rupture. According to these authors, the elongation at the time rupture was related to the relative resistance of the human chordae tendineae at its primary thickness, as opposed to its length [20].

Additional studies had tested radiofrequency energy in vivo to evaluate its effects on the collagen-rich structure of the mitral valve annulus for the mitral regurgitation treatment; one of such study noted a reduction in the mitral valve annulus size [21].

The present study was conducted using normal swine valves and provided important preliminary data, regarding the types and extension of the laser lesions in valvular tissues, although did not recreate chronic valvulopathy. In rheumatic valvar disease, the chords are often thickened and fused, as are the valvular leaflets, which are characterized by chronic inflammation, dense fibrosis, and neovascularization. Moreover, among adults with severe mitral stenosis, densely calcified areas are frequently present. Therefore, the laser energy effects on valves subjected to histological remodeling must be addressed in other future studies.

The international medical literature does not report the HPL utilization for cardiac valves, preventing us from comparing our study with previous papers. This was the first study to assess the behavior of valvular tissue (chordae tendineae) following surgical laser procedure. Such results may possibly guide forthcoming research, regarding how human chordae are affected by mitral insufficiency.

Based on our preliminary data, laser technology may be valuable in the treatment of valvular insufficiency. Additional studies are necessary to verify usefulness of this method. The application of laser technology appears to be promising in regard to cardiovascular surgery armamentarium, particularly involving human pathological valve treatment. Thus, such surgeries would avert the need for artificial replacements or lifetime anticoagulant administration.

Conclusion

In this study, normal, porcine chordae tendineae subjected to an HPL experienced significant reductions in their final lengths and lower stiffness compared to untreated chordae tendineae. Moreover, a histological analysis of the laser treatment specimens demonstrated differences in collagen bundle spatial organization. Such changes in coloration when Masson's trichrome and Picrosirius red staining were applied, which indicate changes in the collagen bundle patterns of the chordae tendineae, following slight changes into tissue temperature.

This study demonstrates that the application of surgical laser is a possible treatment option to be considered for chordae tendineae elongation secondary to mitral valve insufficiency.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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