Article published online: 2021-08-13

Analysis of Nerve Endings in the Superior Labrum-Biceps Complex by Fluorescence Immunohistochemistry and Confocal Laser Microscopy*

Análise de terminações nervosas no complexo labrum-bíceps superior usando imunohistoquímica de fluorescência e microscopia a laser confocal

Maria L. C. Cavalcante[1] Eduardo G. Fernandes[1] José A. Pinheiro Júnior[1] Francisco Vagnaldo F. Jamacaru[2] José Victor de V. Coelho[1] José Alberto Dias Leite[1]

[1] Department of Orthopedics, Universidade Federal do Ceará, Hospital Universitário Walter Cantídio, Fortaleza, CE, Brazil
[2] Department of Pharmacology, Universidade Federal do Ceará, Fortaleza, CE, Brazil

Address for correspondence Maria Cavalcante, MD PhD, Department of Orthopedics, Hospital Universitário Walter Cantídio, Rua Cap. Francisco Pedro, 1395, Fortaleza, CE, 60430-140, Brazil (e-mail: marialuzetecosta@gmail.com).

Rev Bras Ortop 2022;57(5):863–867.

Abstract

Objectives The capsuloligamentous structures of the shoulder work as static stabilizers, together with the biceps and rotator cuff muscles, increasing the contact surface of the glenoid cavity. Free nerve endings and mechanoreceptors have been identified in the shoulder; however, there are a few studies that describe the presence of these nerves in the biceps' insertion. The present study aimed to describe the morphology and distribution of nerve endings using immunofluorescence with protein gene product 9.5 (PGP 9.5) and confocal microscopy.

Methods Six labrum-biceps complexes from six fresh-frozen cadavers were studied. The specimens were coronally cut and prepared using the immunofluorescence technique. In both hematoxylin and eosin (H&E) and immunofluorescence, the organization of the connective tissue with parallel collagen fibers was described.

Results In the H&E study, vascular structures and some nerve structures were visualized, which were identified by the elongated presence of the nerve cell. All specimens analyzed with immunofluorescence and confocal microscopy demonstrated poor occurrence of morphotypes of sensory corpuscles and free nerve endings. We identified free nerve endings located in the labrum and in the bicipital

Keywords
- biceps
- fluorescent antibody technique
- labrum
- mechanoreceptors
- nerve endings
- shoulder

* *Work developed at the Department of Orthopedics, Universidade Federal do Ceará, Hospital Universitário Walter Cantídio, Fortaleza, Ceará, Brazil.*

received June 1, 2020
accepted September 16, 2020
published online August 13, 2021

DOI https://doi.org/10.1055/s-0040-1722579.
ISSN 0102-3616.

Thieme Revinter Publicações Ltda., Rua do Matoso 170, Rio de Janeiro, RJ, CEP 20270-135, Brazil

insertion, and sparse nerve endings along the tendon. Corpuscular endings with fusiform, cuneiform, and oval aspect were identified in the tendon.

Conclusion These findings support the hypothesis that the generation of pain in the superior labral tear from Anterior to posterior (SLAP) lesions derives from the more proximal part of the long biceps cord and even more from the upper labrum. Future quantitative studies with a larger number of specimens may provide more information on these sensory systems.

Resumo

Objetivos As estruturas capsulo-ligamentares do ombro funcionam como estabilizadores estáticos, juntamente com os músculos do bíceps e do manguito rotador, aumentando a superfície de contato da cavidade glenoide. Terminações nervosas livres e mecanorreceptores foram identificados no ombro; no entanto, existem alguns estudos que descrevem a presença desses nervos na inserção do bíceps. Este estudo teve como objetivo descrever a morfologia e distribuição de terminações nervosas utilizando imunofluorescência com *protein gene product* 9.5 (PGP 9.5) e microscopia confocal.

Métodos Foram estudados seis complexos labrum-bíceps de seis cadáveres congelados frescos. Os espécimes foram cortados coronalmente e preparados pelo método de imunofluorescência. Tanto em hematoxilina e eosina (H&E) quanto em imunofluorescência, foi descrita a organização do tecido conjuntivo com fibras paralelas de colágeno.

Resultados No estudo de H&E, foram visualizadas estruturas vasculares e algumas estruturas nervosas, que foram identificadas pela presença alongada da célula nervosa. Todas as amostras analisadas com imunofluorescência e microscopia confocal demonstraram baixa ocorrência de morfotipos de corpúsculos sensoriais e terminações nervosas livres. Identificamos terminações nervosas livres localizadas no labrum, inserção bicipital e terminações nervosas esparsas ao longo do tendão. Terminais corpusculares com aspecto fusiforme, cuneiforme e oval foram identificados no tendão.

Conclusão Esses achados corroboram a hipótese de que a geração de dor nas lesões labrais superiores de anterior a posterior (SLAP, na sigla em inglês) deriva da parte mais proximal do cabo longo do bíceps e ainda mais do labrum superior. Estudos quantitativos futuros com um número maior de espécimes podem fornecer mais informações sobre esses sistemas sensoriais.

Palavras-chave
- bíceps
- técnica de anticorpos fluorescentes
- labrum
- mecanorreceptores
- terminações nervosas
- ombro

Introduction

The superior glenoid labrum serves as the attachment site for the insertion of the biceps' long head tendon, thus being susceptible to the injury produced by its detachment from the glenoid, from anterior to posterior, known as superior labral tear from anterior to posterior (SLAP).[1] Andrews et al.[2] was the first to describe injuries in the superior labrum. These injuries can be frequently observed in young athletes of "throwing sports", being secondary to repetitive micro lesions.[3] It may also occur in association with other traumatic situations, such as primary or recurrent glenohumeral dislocation.[4] Clinically, we observed that, subsequent to anatomical repairs of SLAP lesions, patients presented a prolonged period of pain when compared to those submitted to biceps release procedures.[5–7] The pathophysiological mechanism of pain, present in both lesion and surgical repair as well as in the eventual failure of treatment, has involved the presence of nociceptors. Histological studies of the labral complex have demonstrated the presence of free nerve endings and mechanoreceptors.[8,9] Symptoms such as pain, instability, and a sensation of joint "locking can be attributed to weakening of the proprioceptive reflex.[10] Although reports of SLAP injury are frequent in the literature, recent studies have reported a considerable increase in the number of surgical repairs of these lesions in the last decade.[11] Currently, the advances in antibody markers specific to nerve endings, associated with confocal laser microscopy, allow the visualization and detailing of nerve structures with three-dimensional images.[12–14] The purpose of this investigation was to histologically evaluate the mid-portion of the superior labrum-biceps complex. We hypothesized the

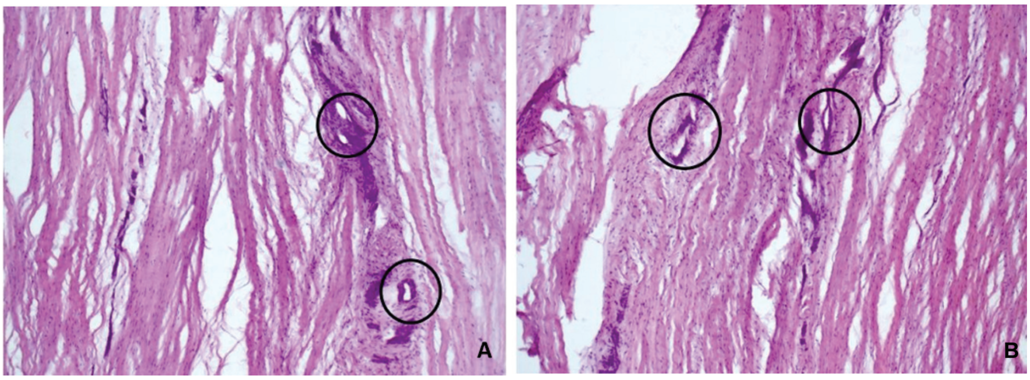

Fig. 1 (A e B) Longitudinal and histological section (10 μm) stained with hematoxylin & eosin. Note the blood vessels (circles) on the biceps tendon.

transition zone between the labrum and the long head of the biceps tendon would contain nerve endings and vessels. We look forward to establish a parallel between our findings and the pathophysiology of the SLAP lesion and tendinopathy of the long head of the biceps.

Methods

We used 6 superior labrum-biceps complex (SLBC) from frozen human cadavers (3 males, 3 females) aged from 20 to 70 years old. Approval for this project was granted and monitored through our institution by the ethics committee (No. 443.172). We used a selective pan-neuronal marker, the pan-axonal protein gene product 9.5 (PGP 9.5) (Thermo Fisher Scientific Inc., Rockford, IL), to highlight sensory innervation. The sections were washed with cold 0.1 M phosphate buffer solution (PBS, Laborclin, Pinhais, PR, Brazil) containing 3% Triton X-100 (TX-100, Inlab, Diadema, SP, Brazil). The tissues were washed and incubated in primary antibody for 2 hours; then, the secondary antibody was conjugated to a fluorescent tag (Alexa Fluor 488 goat anti-rabbit IgG, Thermo Fisher Scientific Inc., Rockford, IL, US). The sections were washed and sealed, and the slides were stored at -70 °C. All 36 sections of the specimens were examined with a confocal laser scanning microscope system (LSM710, Carl Zeiss Microscopy, Jena, Germany). We used hematoxylin & eosin (H&E) and Masson trichrome stain in the mid-portion of the SLBC specimens, and the median nerve was a quality control for immunofluorescence. The results are expressed as means and standard deviation (SD). The data were analyzed using software GraphPad Prism (version 6.0 for Windows, GraphPad Software, San Diego, California, USA, 2015).

Results

Light microscopy analysis of the sections showed easily distinguished the biceps tendon with scarce blood vessels, spaced apart, thin, and slightly wavy (►**Fig. 1, A** and **B**). In the transition portion between the biceps and the labrum, we could observe complex structures, with elongated cells suggesting nerve cells (►**Fig. 2 A, B** and **C**).

Confocal microscopic revealed that free nerve endings through the long head of the biceps tendon, from one to six μm in length, parallel to the collagen bundles, also dissociated from the presence of blood vessels (►**Fig. 3**). In the mid-portion of the SLBC, we identified nerve fibers measuring between 60 and 70 μm in diameter, branching in smaller nerve bundles (►**Fig. 4**).

In addition, the mid-portion of the SLBC, we also observed nerve fibers of smaller caliber, from seven to ten micrometers μm in diameter, close to the vessel, exhibiting peripheral and intraluminal immunoreactivity to PGP 9.5. In the slides submitted to the antigenic recovery technique, the presence of complex nerve endings with variable dimensions, ranging from 150 to 350 μm in length and 80 to 100 μm in width. In the articular face of the samples, next to the labral region and the labrum-biceps transition, we observed axons with between 10 and 20 μm of thickness, and different spatial formats, with predominance of spindle, conical and oval shapes (►**Fig. 5 A, B** and **C**).

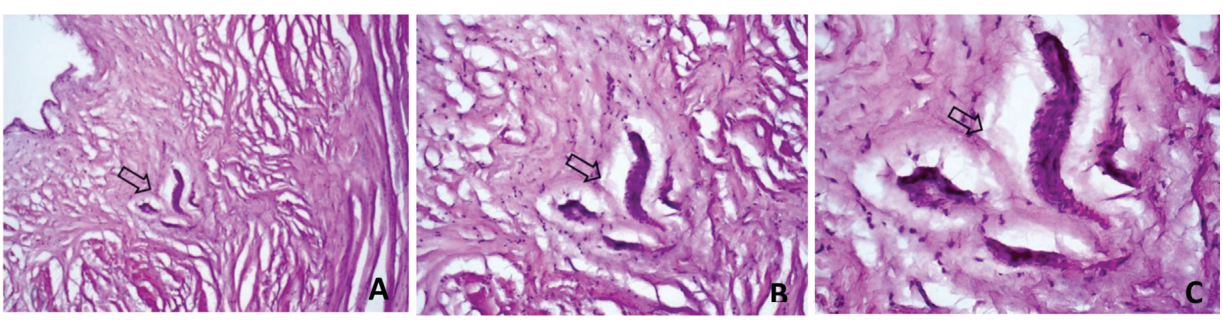

Fig. 2 Fusiform and conical nerves ending of conjunctive tissue in the transition zone between the labrum and biceps. Increase of 50x (A),100x (B) and 200x (C).Longitudinal histological section (10 μm) stained with hematoxylin & eosin.

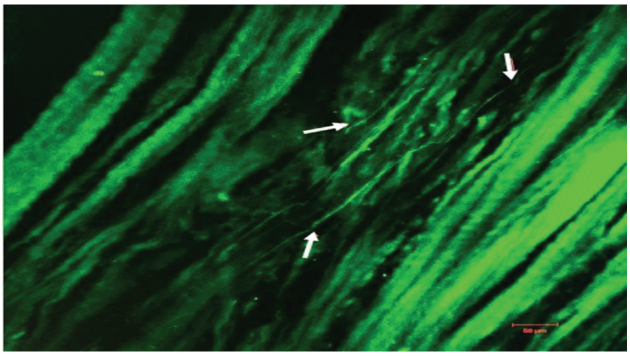

Fig. 3 Section of the biceps tendon (50 μm thick). Observe the free nerve endings (arrows) in the conjunctive tissue, analyzed by confocal laser microscope (immunofluorescence, scale 50 μm).

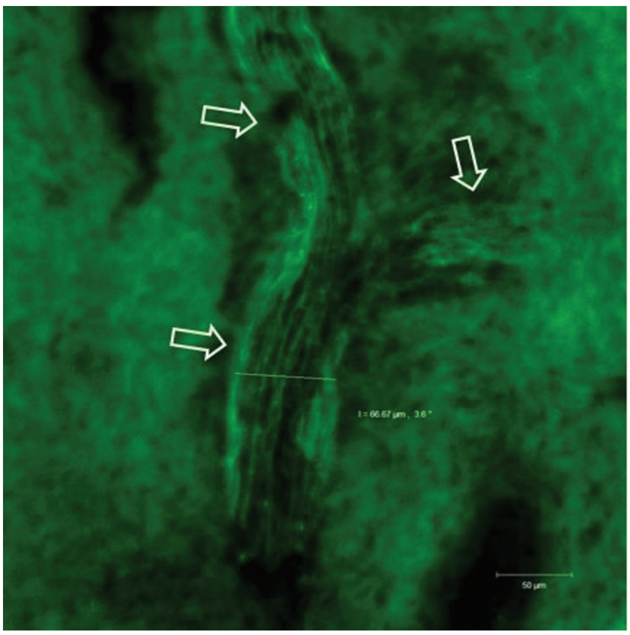

Fig. 4 Transition zone between the labrum and biceps. Note the nerve fiber in the deep layer measuring between 60 and 70 μm in diameter, bifurcating into smaller nerve bundles (immunofluorescence, scale 50 μm).

Discussion

The knowledge of the neuroanatomy of the passive stabilizing structures of the shoulder helps to understand the proprioceptive mechanisms of joint protection and stabilization. The tendon of the long head of the biceps has been studied as a cause of pain in the glenohumeral joint, either in tendinopathies.[15] Alpantaki et al.[16] was the first to study these neural elements in the long biceps' tendon, which he described as containing a large network of sympathetic nerve fibers and sensors, not associated with blood vessels, and with neural distribution predominantly close to its insertion. Our findings were partially compatible with those reported by Alpantaki et al.[16] We have found a few fine nerve fibers, following their own pathways, isolated from the vessels and dispersed along the collagen fiber structure. We also observed, proximally, larger fibers at the labrum-biceps transition around vascular structures, as reported by Boesmueller et al.,[17] who also demonstrated a density of nerves in the proximal segment of the long biceps tendon, similar to the anterior portion of the superior labrum. In agreement with these authors, we observed the presence of neural structures occurring predominantly in the more proximal portion of the biceps tendon. In the samples submitted to the antigenic recovery technique, we observed complex nerve endings near the labral portion and labrum-bicipital transition, which are predominantly distributed in the layers closest to the glenoid joint, making it possibly the first region to be stimulated by contact with the humeral head during shoulder movement. Information exists on the location of neural structures in the upper labrum and bicipital anchor. Among the descriptions, Hashimoto et al.[18] showed the isolated presence of free nerve endings in the labrum and capsular transition; according to Vangsness et al.,[8] we have only free nerve endings; Witherspoon et al.[9] describe only nervous fascicles in the periphery of the anteroinferior and posteroinferior labrum. In relation to the pathophysiological aspect of the SLAP lesion in the shoulder, it seems evident that the bicipital tendon acts as a potential pain generator, having a greater density of neural structures (neurofilaments) in the proximal parts, as observed in others studies.[17,19,20] We identified nerve endings in the three regions (biceps tendon, labrum transition and biceps, upper labrum) of the complex formed by the superior labrum and bicipital insertion, with well differentiated aspects. In the proximal segment of the biceps' tendon, we found fine nerve fibers, without association with vascular structures, and no complex nerve endings were identified. In the transition zone between the labrum and the

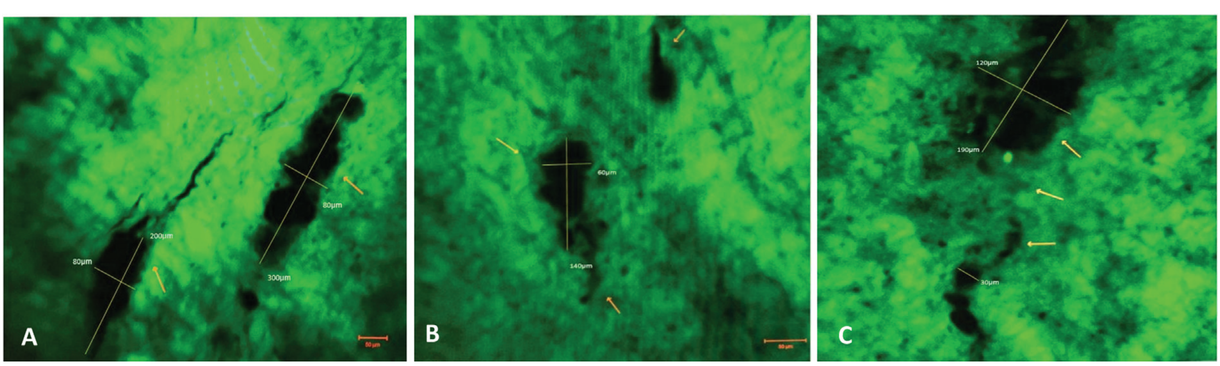

Fig. 5 Nerves endings with different shapes, ranging from 150 to 350 μm in length and 80 to 100 μm in width (Antigenic recovery technique, scale 50 μm).

biceps, we found thick nerve bundles, accompanying arterial blood vessels present in this area. In the labral region and in the labrum-bicipital transition, we found complex nerve endings with oval, conical and fusiform structures.[21] There is a need for new studies to confirm our findings, as well as to accurately identify the mechanoreceptors, including testing other antibodies and cellular markers, thus increasing the number of individuals in the sample, seeking to comprehend all factors related to the interaction of biomechanics and proprioceptive system of the shoulder. The present preliminary study shows the morphology of nerve endings and accurately identifies mechanoreceptors by immunofluorescence. However, the number of specimens and including other antibodies and cellular markers, it would be interesting to compare the anatomical and pathological conditions.

Conclusion

We identified nerve endings in the three regions (biceps tendon, labrum transition and biceps, upper labrum) of the complex formed by the superior labrum and the bicipital insertion, , with well differentiated aspects. In the proximal segment of the biceps' tendon, we found fine nerve fibers, without association with vascular structures, and no complex nerve endings were identified. In the transition zone between the labrum and the biceps, we found thick nerve bundles, accompanying arterial blood vessels present in this area. In the labral region and in the labrum-bicipital transition, we found complex nerve endings, and it was possible to identify them in relation to the spatial format, which consisted of oval, conical, and fusiform structures.

Authors' contributions

Fernandes E. G. was responsible for the conception, design, intellectual and scientific content of the study, acquisition and interpretation of data, and manuscript writing. Cavalcante M. L. C. was responsible for the research, manuscript editing, interpretation of data, and critical review and submission of the manuscript . Jamacaru F. V., Fernandes E. G., Coelho J. V. V., and Leite J. A. D. were involved in the technical procedures.

Financial Support

This work was supported by the Instituto de Pesquisa e Ensino Home, Brasilia, DF, Brazil.

Conflict of Interests

The authors have no conflict of interests to declare.

References

1 Werner BC, Brockmeier SF, Miller MD. Etiology, Diagnosis, and Management of Failed SLAP Repair. J Am Acad Orthop Surg 2014; 22(09):554–565
2 Andrews JR, Carson WG Jr, McLeod WD. Glenoid labrum tears related to the long head of the biceps. Am J Sports Med 1985;13 (05):337–341
3 Snyder SJ, Karzel RP, Del Pizzo W, Ferkel RD, Friedman MJ. SLAP lesions of the shoulder. Arthroscopy 1990;6(04):274–279
4 Maffet MW, Gartsman GM, Moseley B. Superior labrum-biceps tendon complex lesions of the shoulder. Am J Sports Med 1995;23 (01):93–98
5 Boesmueller S, Mayerhofer S, Huf W, Fialka C. Short-term clinical results after arthroscopic type II SLAP repair. Wien Klin Wochenschr 2012;124(11-12):370–376
6 Katz LM, Hsu S, Miller SL, et al. Poor outcomes after SLAP repair: descriptive analysis and prognosis. Arthroscopy 2009;25(08): 849–855
7 Boileau P, Baqué F, Valerio L, Ahrens P, Chuinard C, Trojani C. Isolated arthroscopic biceps tenotomy or tenodesis improves symptoms in patients with massive irreparable rotator cuff tears. J Bone Joint Surg Am 2007;89(04):747–757
8 Vangsness CT Jr, Ennis M, Taylor JG, Atkinson R. Neural anatomy of the glenohumeral ligaments, labrum, and subacromial bursa. Arthroscopy 1995;11(02):180–184
9 Witherspoon JW, Smirnova IV, McIff TE. Neuroanatomical distribution of mechanoreceptors in the human cadaveric shoulder capsule and labrum. J Anat 2014;225(03):337–345
10 Warner JJ, Lephart S, Fu FH. Role of proprioception in pathoetiology of shoulder instability. Clin Orthop Relat Res 1996;(330):35–39
11 Onyekwelu I, Khatib O, Zuckerman JD, Rokito AS, Kwon YW. The rising incidence of arthroscopic superior labrum anterior and posterior (SLAP) repairs. J Shoulder Elbow Surg 2012;21(06):728–731
12 Brodin L, Ericsson M, Mossberg K, Hökfelt T, Ohta Y, Grillner S. Three-dimensional reconstruction of transmitter-identified central neurons by "en bloc" immunofluorescence histochemistry and confocal scanning microscopy. Exp Brain Res 1988;73(02):441–446
13 Chen YG, McClinton MA, DaSilva MF, Shaw Wilgis EF. Innervation of the metacarpophalangeal and interphalangeal joints: a microanatomic and histologic study of the nerve endings. J Hand Surg Am 2000;25(01):128–133
14 Vilensky JA, O'Connor BL, Fortin JD, et al. Histologic analysis of neural elements in the human sacroiliac joint. Spine 2002;27(11): 1202–1207
15 Longo UG, Franceschi F, Ruzzini L, et al. Characteristics at haematoxylin and eosin staining of ruptures of the long head of the biceps tendon. Br J Sports Med 2009;43(08):603–607
16 Alpantaki K, McLaughlin D, Karagogeos D, Hadjipavlou A, Kontakis G. Sympathetic and sensory neural elements in the tendon of the long head of the biceps. J Bone Joint Surg Am 2005;87(07): 1580–1583
17 Boesmueller S, Nógrádi A, Heimel P, et al. Neurofilament distribution in the superior labrum and the long head of the biceps tendon. J Orthop Surg Res 2017;12(01):181
18 Hashimoto T, Hamada T, Sasaguri Y, Suzuki K. Immunohistochemical approach for the investigation of nerve distribution in the shoulder joint capsule. Clin Orthop Relat Res 1994;(305):273–282
19 Joseph M, Maresh CM, McCarthy MB, et al. Histological and molecular analysis of the biceps tendon long head post-tenotomy. J Orthop Res 2009;27(10):1379–1385
20 Tosounidis T, Hadjileontis C, Georgiadis M, Kafanas A, Kontakis G. The tendon of the long head of the biceps in complex proximal humerus fractures: a histological perspective. Injury 2010;41 (03):273–278
21 Tomita K, Berger EJ, Berger RA, Kraisarin J, An KN. Distribution of nerve endings in the human dorsal radiocarpal ligament. J Hand Surg Am 2007;32(04):466–473

