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Published in:
Journal of Orthopaedic Surgery

DOI:
10.1177/2309499018770900

Publication date:
2018

Document Version
Publisher's PDF, also known as Version of record

Article

Journal of Orthopaedic Surgery

Journal of Orthopaedic Surgery
26(2) 1–8

DOI: 10.1177/2309499018770900
journals.sagepub.com/home/osj
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Histology, vascularity and innervation of the glenoid labrum

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Abstract

Background: Although the glenoid labrum has an important role in shoulder stability, little is known about its composition, vascularity and innervation. The aims of this study were therefore to evaluate the histology, vascularity and innervation of the glenoid labrum. **Materials and methods:** Ten glenoid labrum specimens (three male, two female: mean age 81.2 years, range 76–90 years) were detached at the glenoid neck. Following decalcification, sections were cut through the whole thickness of each specimen perpendicular to the glenoid labrum at 12 radii corresponding to a clock face superimposed on the glenoid fossa. Then they were stained using haematoxylin and eosin, a silver nitrate protocol or subjected to immunohistochemistry using anti-protein gene protein 9.5 to demonstrate neuronal processes. **Results:** The labrum was fibrocartilaginous, being more fibrous in its free margin. There was a variable distribution of blood vessels, being more vascular in its periphery, with many originating from the fibrous capsule and piercing the glenoid labrum. Immunohistochemistry revealed positive staining of nerve fibres within the glenoid labrum. **Conclusion:** The glenoid labrum is fibrocartilaginous, being more fibrous in its periphery, and is vascularized, with the anterosuperior aspect having a rich blood supply. Free sensory nerve fibres were also present; no encapsulated mechanoreceptors were observed. The presence of sensory nerve fibres in the glenoid labrum could explain why tears induce pain. It is postulated that these sensory fibres could play a role in glenohumeral joint proprioception.

Keywords

blood supply, glenoid labrum, histology, nerve supply, shoulder joint

Date received: 23 December 2017; Received revised 3 March 2018; accepted: 25 March 2018

Introduction

Descriptions of the constitution of the glenoid labrum are somewhat diverse. It has been described as a fibrous ring or band effectively increasing the depth of the glenoid fossa.[1–3] Others, however, describe it as a cartilaginous structure.[4–9] One study[10] stated that in week 10 of gestation, the glenoid labrum is fibrocellular rather than fibrocartilaginous with collagen fibres; furthermore, it is vascularized with capillaries growing into its free margin by week 12½. In contrast, several studies[11–14] report that the glenoid labrum is composed of dense fibrous tissue with a narrow fibrocartilaginous zone between the articular hyaline surface and the labrum.

Neural receptors of the glenohumeral joint have rarely been observed. Vangsness et al.[15] observed slow adapting

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Ruffini end organs, rapidly adapting Pacinian corpuscles and free nerve ending in the fibrous capsule and the glenohumeral, coracoclavicular and coracoacromial ligaments. Free nerve endings were noted in the peripheral part of the glenoid labrum as well as the subacromial bursae. No encapsulated free nerve endings were detected in the glenoid labrum. However, Guanche et al.[16] reported Golgi's, Ruffini's and Pacini's corpuscles and free nerve ending in 45% of the superior glenohumeral ligament, 42% of the middle glenohumeral ligament, 48% of the inferior glenohumeral ligaments and 47.5% of the fibrous capsule. Only free nerve endings were observed in the long head of biceps tendon and the attached part of the superior glenoid labrum. According to Machner et al.,[17] the proprioceptive sensation of the glenohumeral joint is deficient in post-traumatic anterior glenohumeral instability: Nevertheless, significant improvement in joint proprioception was achieved 18 months following arthroscopic labral repair. The aims of this study were therefore to evaluate the histology, vascularity and innervation of the glenoid labrum.

Materials and methods

Ten glenoid labrum specimens (three male, two female: mean age 81.2 years, range 76–90 years) with no evidence of trauma or degeneration were utilized from five formalin-embalmed cadavers. Sections were cut through the whole thickness of each specimen from the centre of the glenoid fossa perpendicular to the glenoid labrum at 12 radii corresponding to a superimposed clock face. The result was triangular-shaped wedges of the glenoid labrum with the fibrous capsule attached at the periphery. Each wedge was washed in phosphate-buffered saline (PBS) and embedded in paraffin wax using standard techniques. Sections were cut from one non-overlapping radius of each specimen. Sections were stained using haematoxylin and eosin, a silver nitrate protocol or subjected to immunohistochemistry.

Gless–Marsland modification

The tissue is fixed in formalin saline or natural-buffered formalin solution and the paraffin sections cut at 6–8 μm thickness.

Solutions and reagents

1. Twenty percentage of silver nitrate stock solution: dissolve 10 g silver nitrate in 50 ml of distilled water.
2. Ten percentage of formalin solution and must be alkaline: add 10 ml of 37–40% formaldehyde to 90 ml of distilled water.
3. Glees's silver solution: add 30 ml from the 20% silver nitrate stock solution to 20 ml alcohol, then add strong ammonia (0.88) drop by drop with constant agitation until it dissolves and finally add five more drops.
4. Five percentage of aqueous sodium thiosulphate.

Method

1. Rehydrate and clear sections using ethanol and Histo-Clear, then place the sections in distilled water.
2. Place in silver nitrate solution at 37°C for 25–30 min.
3. Rinse in distilled water.
4. Rinse twice and quickly (within 10 s) with 10% formalin solution.
5. Wash off the formalin with Glees's silver solution for 30 s.
6. Pour off the silver solution and flood the slide with formalin solution for 1 min.
7. Examine under microscope, and if sections are not clear, repeat steps 5 and 6.
8. Rinse in distilled water.
9. Place in sodium thiosulphate 5 min.
10. Dehydrate and clear using 95% ethanol, 100% ethanol then Histo-Clear and mount.

Axons and dendrites are stained black, other structures are stained light yellow-brown.

Immunohistochemistry

Anti-protein gene protein 9.5

Anti-protein gene protein 9.5 (PGP 9.5) are neuronal marker antibodies. Slides were prepared and divided into two groups: group I had antigen retrieval using 10% formic acid and group II was a negative control (no primary antibodies). The protocol of the procedure was as follows:

1. Antigen retrieval: 10% formic acid was applied for 10 min.
2. 20 ml PBS + 0.1 ml (0.5%) Triton was mixed well and then added to all groups. This was repeated three times and each time for 5 min. Then wash up gently with PBS.
3. Circulate around the tissue section as close as possible using the hydrophobic pen. This step is critical because the tissue section might become dry and in order to prevent this, PBS should be applied while waiting for the hydrophobic circle to dry.
4. Prepare antibodies diluent: 10 ml PBS + 100 mg albumin bovine (mix well) and then add 10 μl tween 20 (mix well). The antibodies diluent needs to be fresh or no more than a few days old and kept refrigerated.
5. Add 10 μl primary antibodies (PGP 9.5) to 1000 μl antibodies diluent (nota bene [NB]: the antibodies are added to the diluent not vice versa) with the concentration being 1:100.
6. The primary antibodies (1:100) are put on the slides of group I, while group II had the diluent only (negative control). All tissue sections were

confirmed to be completely covered by the solution, after which they were incubated in a humid box in the refrigerator for 2 days.
7. Rinse each slide gently with PBS 3× for 5 min.
8. Apply the secondary antibody (Chemicon AQ132P) goat anti-rabbit horseradish peroxidase (HRP) conjugate diluted 1:200 in dilution buffer for 5 h at room temperature.
9. Rinse each slide gently with PBS 3× for 5 min.
10. Apply DAB solution (0.05% diaminobenzidine tetrahydrochloride plus 0.03% hydrogen peroxide (H_2O_2) in PBS): To make, add 5 mg DAB plus 10 µl 30% H_2O_2 in 10 ml PBS. Then examine the reaction: it takes 2–5 min until the background colour starts to appear.
11. Wash several times with PBS and rinse briefly in water.
12. Dehydrate, clear and coverslip as usual.

Anti-calcitonin gene-related peptide

Anti-calcitonin gene-related peptide (CGRP) is a sensory fibres marker. Slides were prepared and divided into three groups: group I had antigen retrieval using 10% formic acid and group II was a negative control (no primary antibodies). Positive control sections of skin and axillary artery were processed in parallel for quality control. The protocol of the procedure was as follows:

1. Antigen retrieval: 10% formic acid was applied for 10 min.
2. 20 ml PBS + 0.1 ml (0.5%) Triton was mixed well and then added to all groups. It was repeated three times and each time remained for 5 min. Then wash up gently with PBS.
3. Circulate around the tissue section as close as possible using the hydrophobic pen. This step is critical because the tissue section might become dry and in order to prevent this, PBS should be applied while waiting for the hydrophobic circle to dry.
4. Prepare antibodies diluent: 10 ml PBS + 100 mg albumin bovine (mix well) and then add 10 µl tween 20 (mix well). The antibodies diluent needs to be fresh or few days old and kept refrigerated.
5. Add 10 µl primary antibodies (CGPR) to 1000 µl antibodies diluent (NB: add antibodies to the diluent not vice versa) resulting in a concentration of 1:100.
6. The primary antibodies (1:100) were put on the slides of groups I (including the positive control), while group II has the diluent only (negative control). All tissue sections were confirmed to be completely covered with the solution. Then they were incubated in a humid box in the refrigerator for 2 days.
7. Rinse each slide gently with PBS 3× for 5 min.

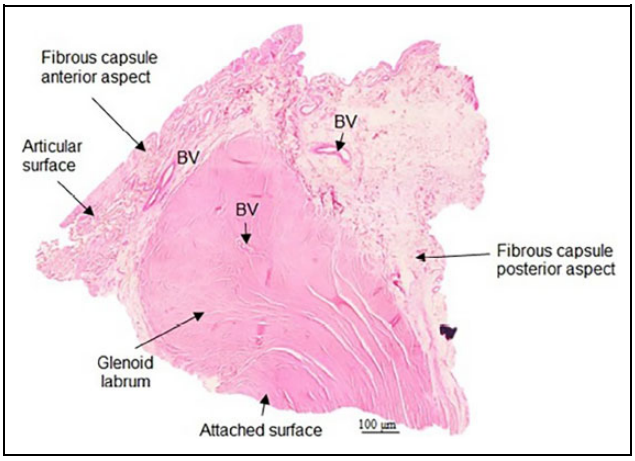

Figure 1. Attachment of the fibrous capsule to the glenoid labrum at 11o'clock left side is shown. BV: blood vessels.

8. Apply the secondary antibody (Chemicon AQ132P) goat anti-rabbit HRP conjugate diluted 1:200 in dilution buffer for 5 h at room temperature.
9. Rinse each slide gently with PBS 3× for 5 min.
10. Apply DAB solution (0.05% diaminobenzidine tetrahydrochloride plus 0.03% H_2O_2 in PBS): To make, add 5 mg DAB plus 10 µl 30% H_2O_2 in 10 ml PBS. Then examine the reaction: it takes 2–5 min until the background colour starts to appear.
11. Wash several times with PBS and rinse briefly in water.
12. Dehydrate, clear and coverslip as usual.

Results

Haematoxylin and eosin

The glenoid labrum was observed to be fibrocartilaginous, being more fibrous in its free margin. It was attached to the articular surface of the glenoid fossa centrally and the glenoid bone peripherally. Some attachments of the labrum to the underlying glenoid bone reached as far as the bone marrow. In different regions, the fibrous capsule split into an internal, covering the internal aspect of the glenoid labrum, and an external, covering the external aspect of the glenoid labrum parts (Figure 1). A variable distribution, in terms of the number and size, of blood vessels was observed in each region, with a greater number of vessels being present in the periphery, many of which arose from the fibrous capsule to pierce the glenoid labrum.

Silver nitrate

Sections stained with silver nitrate revealed nerve fibres scattered within the glenoid labrum (Figure 2).

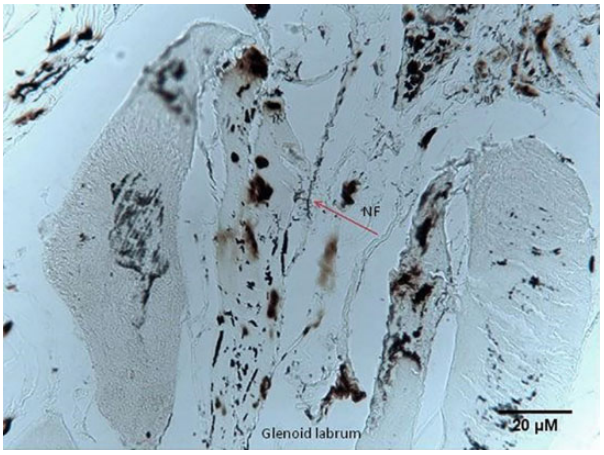

Figure 2. Glenoid labrum stained with silver nitrate showing nerve fibres (arrow).

Immunohistochemistry

PGP 9.5, which is known to label nerve fibres, positively stained numerous nerve fibres in the glenoid labrum (Figure 3). Negative control slides, excluding the primary antibody, did not show any nerve fibre staining. CGRP, a specific marker for sensory nerve fibres, labelled such fibres within the glenoid labrum (Figure 4). Blood vessel sections used as a positive control clearly showed positive labelling for sensory nerve fibres (Figure 5). Negative control sections, lacking primary antibody, showed no evidence of nerve fibre labelling.

Discussion

The exact morphology of the glenoid labrum is a matter of some debate. It is described as a fibrous ring or fibrous band,[1–3] whereas others[4–9] state that it is a cartilaginous structure. However, Nazir et al.[10] report that it is fibrocellular rather than fibrocartilaginous with collagen fibres. In contrast, other studies[11–14] are of the opinion that the glenoid labrum is composed of dense fibrous tissue with a narrow fibrocartilaginous zone between the articular hyaline surface and the glenoid labrum. One study[13] also added that cellularity and vascularity of the labrum and the transitional zone increased with age. Ockert et al.[18] confirmed that the glenoid labrum has a circumferentially avascular fibrocartilaginous zone constituting up to one-third of the glenoid labrum in cross section, the rest being dense fibrous tissue. Arai et al.[19] reported that the composition of the superior glenoid labrum is collagen fibres that run circumferentially along with some elastic fibres. Using electron microscopy, the glenoid labrum found to have three layers of collagen: the first is a superficial layer consisting of a thin reticular fibrillar network, the second is a stratified layer while the third layer consisted of densely arranged bundles of fine fibrils which ran parallel to each other but oblique to the glenoid rim.[20] Hill et al.[21] reported three glenoid labrum zones: the first is a superficial mesh of multidirectional fine fibrils, the second is a loose orientation of fibres characterized by its vascularity and noted to be most common in the anterosuperior region compared to other regions and the third is the central core which consists of large dense fibre bundles circumferentially oriented and avascular. Using light microscopy, the current study agrees with Ockert et al.[18] stating that the glenoid labrum is fibrocartilaginous, becoming more fibrous in the periphery. It supports Hill et al.[21] in that the whole glenoid labrum is vascular, with the anterosuperior aspect of the glenoid labrum having a rich blood supply.

Two studies[14,21] reported that the glenoid labrum is attached to the underlying glenoid bone by vertical and oblique interweaving fibres with associated Sharpey's fibres anchoring onto the superficial bony surface of the glenoid. Whereas the attachment to the underlying hyaline cartilage is by finger-like processes via foramen in the superficial aspect of the hyaline cartilage in association with Sharpey's fibres. The interdigitating anchoring fibres and Sharpey's fibres attach to the underlying glenoid bone and cartilage in different orientations supporting the idea that the glenoid labrum is subjected to various multidirectional forces. They have added that collagen fibres of the glenoid labrum at the labrum–articular cartilage interface were not very dense between 11 and 4 o'clock and were associated with a loose or incomplete attachment of the glenolabral junction. A complete attachment between the glenoid labrum and the underlying articular cartilage between 5 and 11 o'clock was observed. The glenoid labrum region between 10 and 12 o'clock was attached to the apex of the glenoid rim, while in the other regions of the clock face the articular cartilage did not extend to the glenoid edge because the glenoid labrum had a bony foundation and was covered by the glenoid edge. Clinically, a series of studies[22,23] reported that injury of the superior and anterior aspect of the glenoid labrum such as Bankart and superior labral tear from anterior to posterior (SLAP) lesions have high incidence in patients with shoulder dislocation. The current study agrees, observing that the glenoid labrum attaches to the underlying articular surface centrally and was anchored to the underlying glenoid bone peripherally, reaching to bone trabeculae in some regions. Grossly, the superior half of the glenoid labrum (mainly from 11 to 2 o'clock) was incompletely attached to the underlying articular surface and glenoid bone. These findings could explain why with shoulder dislocations have a high incidence of Bankart or SLAP lesions.

The consistency of the glenoid labrum ranged from rubbery to firm. Shoulders of individuals in their fifth decade at the time of death had a glenoid labrum that was thin and virtually absent. The glenoid labrum was sparsely vascularized without any configurative pattern of distribution and the vascularity was observed to decrease with age.[24] In contrast, one study[25] reported that the vascular channels

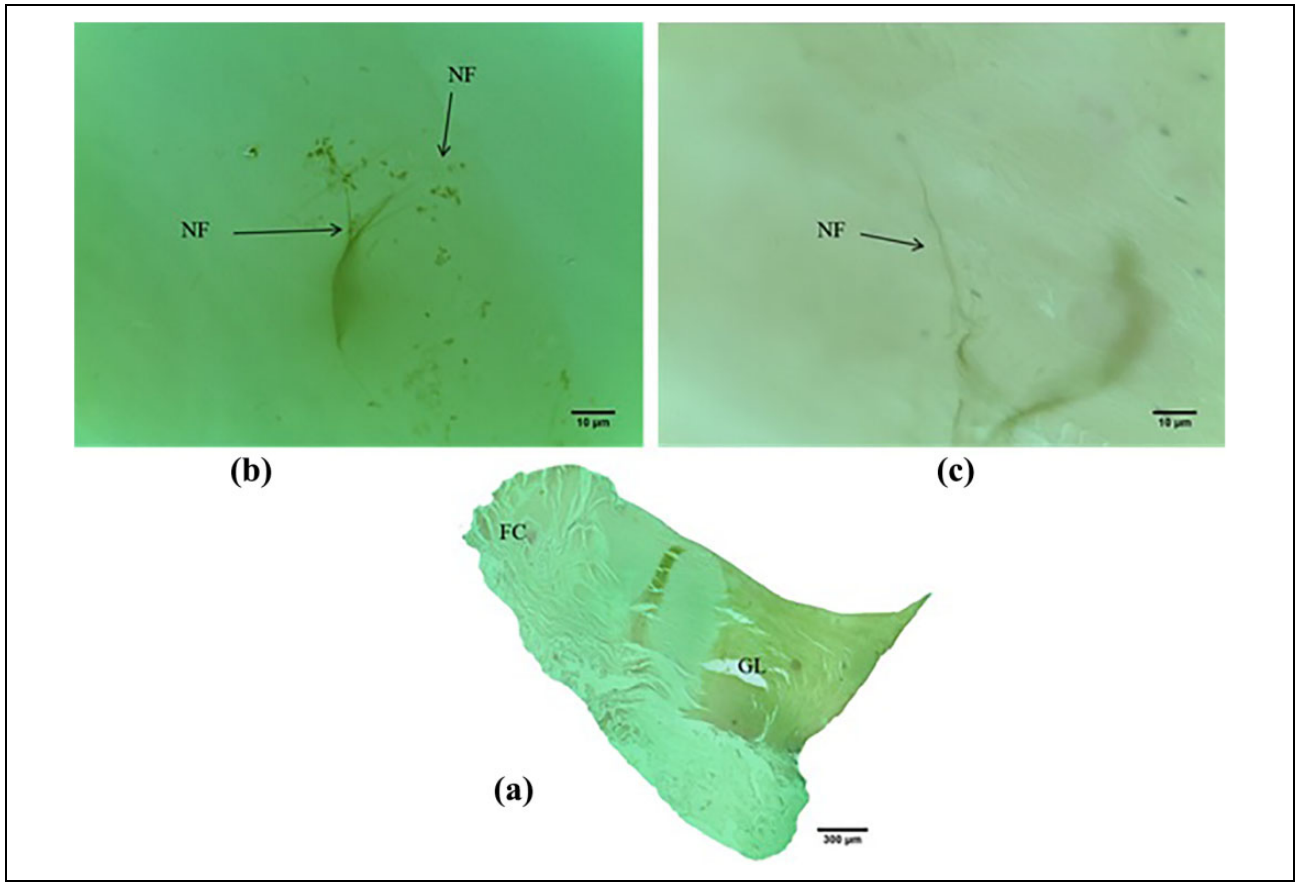

Figure 3. (a) GL and FC; (b) and (c) NFs within the GL. GL: glenoid labrum; FC: fibrous capsule; NF: nerve fibre.

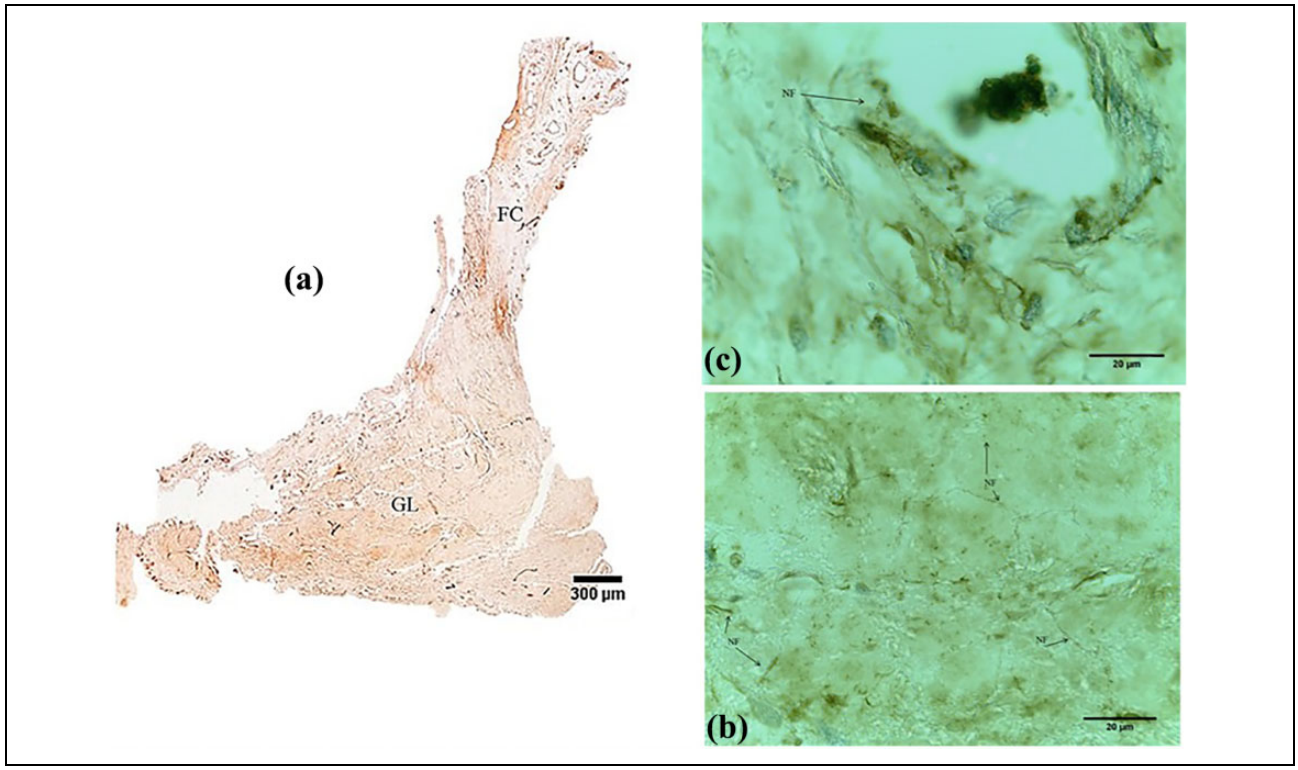

Figure 4. (a) GL and FC. (b) NFs inside the GL. (c): Blood vessel with nerve fibres in its wall. GL: glenoid labrum; FC: fibrous capsule; NF: nerve fibre.

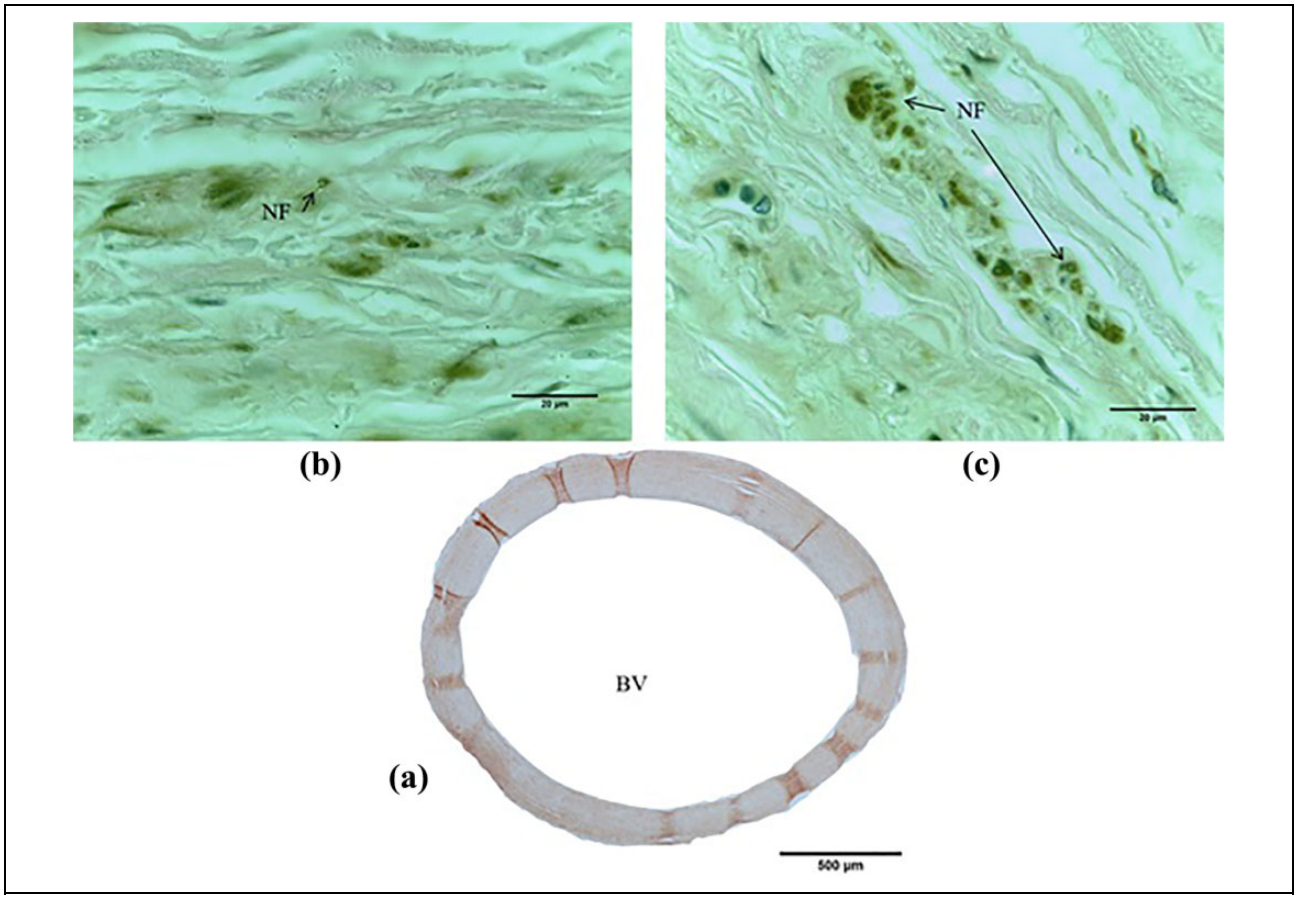

Figure 5. (a) Transverse section of a BV positive control, (b) NFs and (c) NFs. BV: blood vessel; NF: nerve fibre.

proliferating inside the glenoid labrum and glenoid bone increased with gestational age. The current study observed that the consistency of the superior half of the glenoid labrum was rubbery in 97.86% ($n = 137$) and firm in 2.14% ($n = 3$) of specimens, whereas the entire inferior half was firm. Assuming that the shape and size of the glenoid labrum is linked to its consistency, the superior half of the glenoid labrum was triangular and thicker giving it the rubbery appearance while the inferior half was rounded and smaller in size, making it firm.

Neural receptors of the glenohumeral joint have rarely been observed; the first report was by Vangsness et al.[15] using a modified gold chloride stain. In the fibrous capsule, there were slow adapting Ruffini end organs, rapidly adapting Pacinian corpuscles and free nerve endings in the glenohumeral, coracoclavicular and coracoacromial ligaments. Free nerve endings were noted in the present study, but could not be confirmed, in the peripheral part of the glenoid labrum as well as the subacromial bursae. The number of neural receptors was not quantified. The encapsulated mechanoreceptors could not be detected in the glenoid labrum. However, Guanche et al.,[16] using the same stain as Vangsness et al.,[15] reported four neural receptors, these being Golgi, Ruffini and Pacini corpuscles as well as free nerve endings in 45% of the superior glenohumeral ligament, 42% of the middle glenohumeral ligament, 48% of the inferior glenohumeral ligaments and 47.5% of the fibrous capsule. Only free nerve endings were revealed in the long head of biceps tendon and the attached part of the superior glenoid labrum. According to Machner et al.,[17] the proprioceptive sensations of the glenohumeral joint were deficient in post-traumatic anterior glenohumeral instability: A significant improvement in joint proprioception was achieved 18 months following arthroscopic labral repair, which raises the question of whether the sensory nerve fibres of the glenoid labrum play a role in the proprioception of the glenohumeral joint. The current study augments, using silver nitrate stain, the findings of Vangsness et al.,[15] Guanche et al.[16] and Machner et al.[17] and for the first time, using immunohistochemistry, confirms that there are free sensory nerve fibres in the glenoid labrum. No encapsulated nerve endings were observed. As many free nerve endings act as nociceptors, this finding emphasizes that the tears of the glenoid labrum could induce pain; furthermore, if the glenoid labrum is enriched with sensory fibres, it could play a role in glenohumeral joint proprioception.

A series of studies[26–29] has reported shoulder pain as a sign of glenoid labrum tear, but in contrast nothing has been reported about the glenoid labrum type of nerve fibres. A major advantage of the current study, in investigating the

type of nerve fibres of the glenoid labrum, was to use both PGP 9.5 and CGRP antibodies. PGP 9.5 antibody has been used in several previous studies and specifically in the identification of neuronal cell bodies and nerve fibres of all types in the peripheral tissue.[30–38] Whereas CGRP antibody is generally considered as neurotransmitters of nociceptive sensory neurons.[39] The staining pattern observed in the current study is consistent with the previous studies that used the same PGP 9.5 and CGRP antibodies.[30–39] The correlation between the number of nerve fibres of the glenoid labrum and glenoid labrum region, age, sex or side is still unknown. Further study is therefore recommended to evaluate the association between the number of the glenoid labrum nerve fibres and glenoid labrum region, age, side and sex.

Conclusion

The glenoid labrum is a fibrocartilaginous structure that becomes more fibrous peripherally. It is attached to the underlying articular surface centrally and anchored to the underlying glenoid bone peripherally, reaching to bony trabeculae in some regions. The whole of the glenoid labrum is vascular, especially the anterosuperior aspect that has a rich blood supply. Several blood vessels were observed arising from the fibrous capsule to supply the glenoid labrum. Occasionally, the glenoid labrum was observed to reach bony trabeculae through the periosteal layer, providing additional blood supply. Using a silver nitrate stain and immunohistochemistry, free sensory nerve fibres were observed in the glenoid labrum; however, no encapsulated nerve endings were observed. The presence of sensory nerve fibres could explain why tears of the glenoid labrum induce pain. Furthermore, these sensory nerve fibres could play a role in glenohumeral joint proprioception.

Authors' note

All authors have participated in the research for this article.

Acknowledgements

The authors express their thanks to the donors and their families, without them this study would not have been possible. The authors also thank both the Centre for Anatomy and Human Identification, University of Dundee and the Centre of Anatomy, University of Zawia.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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