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Immunohistochemical analysis of mechanoreceptors in transverse acetabular ligament and labrum: a prospective analysis of 35 cases

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Objective: The labrum and transverse acetabular ligament (TAL) are classically described as distinct anatomical structures with abundant mechanoreceptors and free nerve fibers. They deepen the joint and act as natural barriers against dislocation, thus providing additional stability. We hypothesized that severe coxarthrosis leads to elimination of labrum and TAL mechanoreceptors. This study evaluated the microscopic anatomy of the labrum and TAL and specifically investigated the neurological status of these structures.

Methods: Labral and TAL specimens from 35 consecutive patients with coxarthrosis undergoing total hip arthroplasty were dissected into a total of 4 specimens per hip. Formaldehyde-fixed specimens were evaluated for the presence of mechanoreceptors and free nerve endings by neurofilament protein and S-100 protein immunohistochemistry and microscopy.

Results: Mechanoreceptors were identified in the labral (2.3/high power field) but not TAL specimens, with a sharp delineation between the tissues. In contrast, both labrum and TAL showed good vascularity with abundant free nerve fibers within fibrous connective tissue (mean, 2.6 sensory fibers/high power field vs 3.1/high power field, respectively).

Conclusion: This study demonstrated that the TAL lacks mechanoreceptors. However, TAL and labrum free nerve fibers suggest potential roles as hip pain generators.

Keywords: Coxarthrosis; labrum; mechanoreceptors; transverse acetabular ligament.

Total hip arthroplasty (THA) is an effective surgical intervention which alleviates pain and improves quality of life. Pain is the main symptomatic indication for THA. Joint pain is thought to originate from the stimulation of A-delta mechanoreceptors and group C polymodal nerve endings present in the joint synovium and associated structures including the capsule, ligaments, and muscles.[1] Another source of hip pain is "migraine" of the femoral head—i.e., pooling of the venous drainage due to fibrous thickening of the capsule, resulting in tension stimula-

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tion of the receptors surrounding the hip joint.[2,3] Joint mechanoreceptors have been extensively examined since the first description of the Pacini corpuscle by Rauber in 1874.[4–6] Based on numerous animal studies of neurological structures, Ruffini, Pacinian, and Golgi-type mechanoreceptors have been described.[7–11] These studies demonstrated that rapidly adapting Pacinian corpuscles are sensitive to pressure and vibratory stimuli, whereas slowly adapting Ruffini receptors are sensitive to stretching.

Similar to other human joints, the hip joint has afferent and efferent feedback systems that propagate signals from peripheral mechanoreceptors and free nerve endings to convey proprioceptive information to the cortex.[12] Hip ligaments, including the labrum and transverse acetabular ligament (TAL), have mechanoreceptors that provide feedback to the central nervous system to avoid joint injury. The term for this feedback is proprioception. Severe coxarthrosis is known to not only affect the joint cartilage but also the supporting tissues such as the joint capsule, the labrum, and ligaments. These mechanoreceptors are reported to decrease concurrent with knee osteoarthritis.[13,14]

Along with other paracoxal structures, the labrum and TAL provide additional stability to the hip joint. TAL acts as a tension band between the posteroinferior and anteroinferior aspects of the acetabulum during joint loading, whereas the labrum deepens the socket and increases the acetabular contact area.[15] Although a cadaver study showed the presence of some TAL and labral mechanoreceptors, clinical evaluation of the fate of TAL and labrum mechanoreceptors in coxarthrosis has never been reported.[16]

In this study, we attempted to quantify the different neural elements in labrums and TALs obtained from osteoarthritic joints undergoing THA. A through immunohistomorphometric analysis was performed, which is more reliable and easier to conduct than traditional methods of histological staining.[17] We hypothesized that severe coxarthrosis leads to annihilation of labrum and TAL mechanoreceptors.

Patients and methods

The Institutional Review Board approval was obtained prior to study initiation. Informed consent was obtained from 29 female and 6 male patients with a mean age at operation of 66.4 years (range: 55–68 years) undergoing total hip arthroplasty. All patients had end-stage primary coxarthrosis, and patients with secondary arthrosis were not included into the study. All osteoarthritic hips had a near-normal collodiaphyseal angle, and there were no more than 2 cm of leg length discrepancy. A total of 35 specimens were obtained from 35 consecutive patients.

All patients underwent the same operative procedure. A posterolateral approach was utilized, and en bloc resection of the acetabular labrum and TAL was performed. The labral specimens were then marked according to their anatomical locations as anterior, superolateral, or posterior. All labral and TAL specimens were preserved in 10% formaldehyde. Fixed tissue was sectioned and processed for histological immunohistochemistry as previously described.[18] After incubation with 3% normal horse serum to block nonspecific binding, tissue sections were incubated overnight at 4°C in a humid chamber with commercially available mouse monoclonal antibodies against either neurofilament protein (NFP) or the S-100 protein. These markers were used to label the central axon, the axonic-related Schwann cells, and the perineural-related mechanoreceptor cells. The presence and/or absence of mechanoreceptor organs was evaluated under light microscopy (magnification ×400) using the point-counting method.

The classification system of Freeman and Wyke was used to determine the types of receptors present.[7] Using this classification system, there are 4 types of nerve endings: type I, Ruffini corpuscles that are low-threshold and slow adapting; type II, Pacini corpuscles that are low-threshold and fast adapting; type III, Golgi tendon organs that are low-threshold and slow adapting; and type IV, that are the free nerve endings of high-threshold nociceptors. Neural end organ concentrations in the TAL and zone-specific labral specimens were calculated as the overall mean number of neuron fibers per high power field (hpf) under light microscopy (magnification ×400).

Results

No mechanoreceptors were identified in the TAL specimens harvested in this study. Unmyelinated free nerve ending were classified as sensory fibers. Histologically, H&E-stained labral zones and transverse acetabular ligamentous tissue sections showed good vascularity with abundant free nerve fibers (mean 2.6 sensory fibers/hpf vs 3.1 sensory fibers/hpf, respectively) within fibrous connective tissue (Figures 1a, b). There were also areas of calcification and thick collagen fiber bundles within these areas. Although immunohistochemical analysis showed no morphologically normal forms of mechanoreceptors in any TAL tissue samples, the labrum contained a mean of 2.3 mechanoreceptors/hpf (Table 1).

Discussion

This study identified a sharp delineation in mechano-

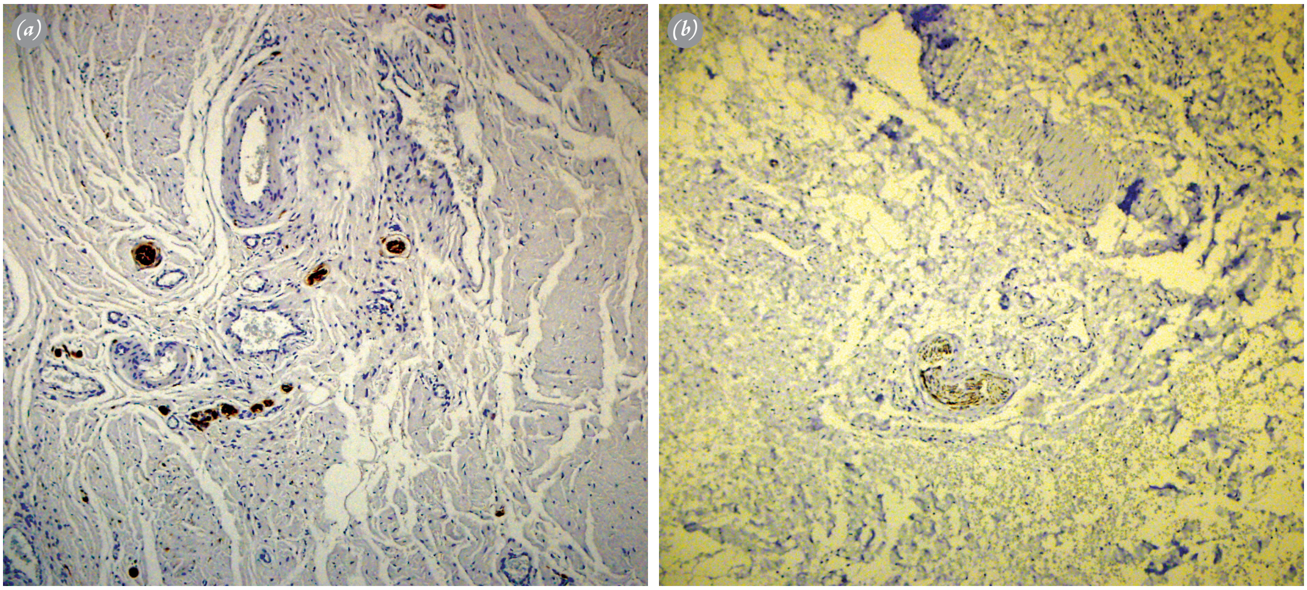

Fig. 1. **(a)** Photomicrograph of the anterior labrum illustrating S-100 positive mechanoreceptors. **(b)** Photomicrograph of the transverse acetabular ligament with neurofilament protein-positive peripheral nerve fibers without visible mechanoreceptors. Tissues were from a 67-year-old woman. (Magnification ×400.) [Color figures can be viewed in the online issue, which is available at www.aott.org.tr]

receptor presence between the labrum and TAL; there was an absence of the intraligamentous mechanoreceptor component in the TALs of hips affected of osteoarthritis. The osteoarthritic degenerative process leads to scarring and subsequent stiffening of the TAL. The lack of TAL mechanoreceptors may well be explained based on the degenerative process involving both pericoxal ligaments and the joint. In addition to the vascularity of the periarticular structures, the neurological architecture must also be considered for degenerative conditions. In many animal studies, this neurological system was examined, and Ruffini, Pacinian, and Golgi-type mechanoreceptors were described.[7,9,11,19] In a therapeutic study, Moraes et al. compared the density of nerve endings in both patients with hip arthrosis and healthy cadavers and found a significant reduction of mechanoreceptors in the arthrosis cohort.[12] This comparative study is the only one in the literature to investigate mechanoreceptor status in patients with coxarthrosis. The study also found a greater loss of nerve endings among those that adapted rapidly to the groups with arthrosis and concluded that hip joint arthrosis is accompanied by a considerable reduction in the number of associated mechanoreceptors. In the present study, we found no mechanoreceptors in the TALs of the hip joint, but we found abundant free nerve fibers in both the TAL and labrum.

In a cadaver study conducted by Gerhardt et al., nerve fibers and free nerve endings were evaluated in the hip capsule, acetabular labrum, ligamentum teres, and TAL of healthy cadavers.[16] The authors used a modified gold staining technique and noted 1.9 mechanoreceptors/hpf, with a preponderance of Ruffini corpuscles and 2.2 sensory fibers/hpf in the TALs. The concentrations of free nerve fibers both in both the TAL and labrum were higher in the present study than was previously reported.[16] This difference could be explained at least in part by the different staining procedures used for identification. This previous study analyzed formalin-fixed and paraffin-embedded specimens stained with aqueous gold chloride. Although the neurological characterization of the whole hip joint was excellent, the effect of osteoarthritis on the quantity of receptors was not report-

Table 1. Overall mean number of sensory fibers and mechanoreceptors in transverse acetabular ligament and acetabular labrum specimens from patients undergoing total hip arthroplasty.

	Anterior labrum	**Superolateral labrum**	**Posterior labrum**	**Transverse acetabular ligament**
Sensory fibers / High power field	3.7	1.8	2.5	3.1
Mechanoreceptors / High power field	3.3	1.5	2.1	–

ed despite 3 of 8 cadavers showing severe osteoarthritis. Also, the gold staining technique has been found to be less efficacious for staining thin neuronal structures, and is nonspecific in that it also stains the vasculature and other structures containing elastin and collagen.[20,21]

Joint mechanoreceptors are essential for proprioception.[22] Active hip joint stability is achieved through proprioceptive nerve ending signaling and damage to these receptors may be responsible for the loss of feedback to the central nervous system that can lead to joint instability.[9,16,23] One conclusion from this finding is that preserving these ligaments during surgery avoids the loss of proprioception; however, the present study revealed a complete loss of receptors despite the presence of abundant free nerve fibers in the TAL. Although extremely rare, some cases at least experience disturbing postoperative pain of unknown origin. This pain may be explained by positive stimuli from the receptors and free nerve fibers to new pressure and capsule stretching. Similar to the knee joint, the hip joint may have deep sensitivity originating from this neural system and functioning receptors in hip ligaments other than TAL may contribute to this form of pain. Although one cannot conclude in the strict sense that mechanoreceptors are the source of this pain, Assimakopoulos et al. proposed that the denervation effects of meniscectomy may induce pain relief and that this technique can be adapted to the hip joint.[24]

The present study has a number of assumptions and limitations. First, there were no age-matched, normal control subjects without joint degeneration enrolled for comparison. Second, the study used immunohistochemical staining for structure identification, whereas other studies used the Zimny technique or the Bodian method to evaluate free nerve ending and mechanoreceptor concentrations. However, Bali et al. have reported that immunohistochemical staining yields superior and reliable data compared to more traditional staining methods.[17] The simultaneous use of 2 different staining techniques should be more influential for determining the optimal methods for examining these neural structures. Third, only one cytopathologist examined the samples, which may have introduced bias. Fourth, a quantitative assessment of the labrum and TAL blood nourishment would be appropriate; however, for this evaluation, immunohistochemical staining to detect laminin should be used. This assessment would likely be more valuable for restoration of peripheral labral tears. Finally, only intraarticular structures were examined. Examination of all periarticular structures, including the hip joint capsule, might provide a more rounded assessment of the relationship between coxarthrosis and mechanoreceptor expression. Further studies of all whole hip ligaments with a larger number of specimens in both arthritic and non-arthritic subjects with functional analysis data may provide more conclusive information on the clinical relevance of TAL mechanoreceptors.

Our observations suggest that the TAL can retain its microarchitecture when it is left intact during THA, but without information from the surgical cases, it may be difficult to draw any conclusions as to what parameters are involved in its successful retention or how it may be functioning. Although our study was not conducted to answer the question of whether or not the TAL should be retained at surgery, the ligament appears to have lost its mechanoreceptors during osteoarthritis progression and become totally absent by end-stage disease. TAL can be completely excised after the acetabular cup is implanted, since previous publications suggest using TAL as an anatomical landmark for cup orientation.[25–28] Although TAL can aid acetabular cup orientation, TAL can be difficult to identify in some patients with advanced degenerative disease and with dysplastic hips showing increased sagittal tilt.[29]

In conclusion, the TAL identified in the current study lacks the pressure and proprioceptive receptors that are present in other stabilizing hip structures such as the labrum. However, the presence of free nerve fibers suggests that TAL may act as a pain generator and should be excised after cup implantation.

Conflics of Interest: No conflicts declared.

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