Histological changes and neural elements in the subacromial bursa on patients with rotator cuff tear
Pilot study

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Abstract

Objective: This study aimed to investigate the neural elements of the subacromial bursa (SAB) in rotator cuff tears.

Materials and methods: Twenty patients with rotator cuff tears were recruited, and their visual analog scale (VAS) score, duration of symptoms, and range of motion (ROM), including flexion, external rotation, and internal rotation were evaluated. Tear size was measured using magnetic resonance imaging (MRI). The SAB specimens obtained during arthroscopic rotator cuff repair were studied using routine hematoxylin and eosin staining and immunohistochemistry (S-100 protein and PGP 9.5 protein). The SAB specimen for the control group was obtained from 2 fresh cadavers and 2 patients with acute humeral shaft fracture. The Mann-Whitney U test was applied to assess the difference between histological findings of the rotator cuff tear group and control group. The correlation between the histological findings and clinical features was evaluated using the Spearman correlation coefficient.

Results: The mean duration of symptom was 10.2 ± 6.4 months. The preoperative average VAS score was 2.9 ± 1.2. The degrees of preoperative ROM in forward flexion and external and internal rotations were 143.8 ± 19.5, 49.5 ± 23.1, and –4.3 ± 4.2, respectively. The tear was 2.0 ± 0.9 cm. For histological findings, the number of neural elements per low power field in the rotator cuff tear group was significantly less than the control group in both immunohistochemical stainings (S-100: 0.5 ± 0.7 vs 2.8 ± 0.5, p < .01; PGP 9.5: 0.4 ± 0.7 vs 3.5 ± 0.6, p < .01). During the correlation analysis, the number of neural elements in the PGP 9.5 staining was negatively correlated with the ROM in forward flexion and external rotation.

Conclusion: This study revealed that chronic rotator cuff tears may induce degeneration of neural elements in SAB.

Abbreviations: H&E = hematoxylin and eosin, HRP = horseradish peroxidase, IL = Interleukin, LPF = low power field, ROM = range of motion, SAB = subacromial bursa, VAS = visual analogue scale.

Key Words: bursa, bursectomy, histology, nerve, rotator cuff tear, shoulder

1. Introduction

The subacromial bursa (SAB), which is positioned under the deltoid muscle and coracoacromial arch, is the largest bursa of the shoulder joint. For lubrication of the shoulder joint, SAB plays a role in decreasing the friction between the coracoacromial arch and rotator cuff tendon.[11] Several studies have been conducted to determine whether the SAB should be removed during arthroscopic surgery for rotator cuff tears. Some authors describe SAB as a pathologic tissue that should be removed, as it may cause an inflammatory response that increases pain.[12–17] On the other hand, other authors who claim that SAB is a vascularized connective tissue that covers rotator cuff tears are opposed to removing SAB because proliferating cells found in the fragmented tendons can play a role in remodeling and healing of the rotator cuff tendon.[8–11] However, most studies related to subacromial bursectomy focus only on pain that can be caused by various cytokines and growth factors, and few studies focus on neurohistology of SAB. Moreover, few studies have investigated the neural elements of the bursa in the normal shoulder group or patient group with rotator cuff tears. However, no study has compared the 2 groups. Therefore, to investigate the state of neural elements in the bursa of rotator cuff tears, research with a control group is necessary.

C-HC and DGK have contributed equally to this work.

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Considering the importance of investigating the neural elements of SAB in the rotator cuff tear group and healthy control group and in light of the aforementioned issues, we aimed to investigate the neural elements of SAB in rotator cuff tears and to determine the clinical relevance of rotator cuff treatment based on histological results.

2. Material and methods

2.1. Demographics and inclusion and exclusion criteria

A total of 20 patients (10 men and 10 women, age: 61.9 ± 6.9 years) with rotator cuff tears were enrolled in this study for a period of 2 months between March and May 2017. The cases were classified as small (n = 5), medium (n = 9), and large (n = 6) tears, according to the Cofield classification of tear size. The exclusion criteria were as follows: previous shoulder operation, shoulder joint dislocation, fracture around the shoulder joint, such as proximal humeral and acromial fractures, shoulder joint infections, systemic inflammation including rheumatic arthritis, and open rotator cuff repair. The inclusion criteria were as follows: rotator cuff tear involving the supraspinatus tendon, full-thickness tear (small-to-large tear), and arthroscopic rotator cuff repair, including subacromial bursectomy. SAB specimens from the study group were obtained during arthroscopic rotator cuff repair, while those from the control group were obtained from 2 fresh cadavers (1 man and 1 woman, 62 and 64 years, respectively) that did not meet the exclusion criteria and 2 patients with acute humeral shaft fracture (1 man and 1 woman, 76 and 77 years, respectively). In a case of acute fracture, the bursa specimen was obtained within days after the fracture; therefore, there was no enough time to induce changes in the neural specimen in the bursa. Thus, we used the bursa as a control. This study was approved by the Daegu Catholic University Research Ethics Committee (IRB no.: CR-20-113-1) and was conducted in accordance with the Declaration of Helsinki. Informed consent was not required due to the retrospective nature of the study.

2.2. Clinical evaluation

Clinical outcomes were evaluated according to the visual analog scale (VAS) score for pain, duration of symptoms, and range of motion (ROM) in flexion, and external and internal rotations. The degree of internal rotation was measured by the thumb tip location on the spinal segments, and the highest points on the affected and unaffected sides were compared.

2.3. Radiological assessment

Magnetic resonance imaging (MRI) was performed using a 1.5-T MRI scanner (SIGNA; GE Healthcare, Chicago, IL). Fast spin-echo T2-weighted sequences and fat-suppressed fast spin-echo T2-weighted sequences were used in the sagittal and coronal oblique planes. The size of the rotator cuff tear was measured in 2 plane images (sagittal and coronal obliques), and the mean value of the greatest diameter measured in each image was used. Rotator cuff tear was determined by the high-signal area within the tendon or a defective tendon filled with fluid on a T2-weighted image.

2.4. Histologic assessment

Subacromial bursa samples (2 × 2 mm) were excised during the arthroscopic rotator cuff repair. These samples were fixed in 10% buffered formaldehyde, embedded in paraffin; 5 μm sections were processed for routine hematoxylin and eosin (H&E) staining and immunohistochemical studies. The SAB specimens were studied using routine H&E and immunohistochemistry (S-100 protein and PGP 9.5 protein). Immunohistochemistry was carried out on 5 μm paraffin-embedded tissue sections using the Bond Polymer Intense Detection System (Leica Microsystems, Wetzlar, Germany), according to the manufacturer’s instructions with minor modifications. In brief, deparaffinization of formalin-fixed, paraffin-embedded tissue sections using Bond Dewax Solution (Leica Microsystems) was followed by antigen retrieval using Bond ER Solution (Leica Microsystems) for 30 minutes at 100°C. To quench the endogenous peroxidase, the sections were immersed in hydrogen peroxide for 5 minutes and incubated for 15 minutes at room temperature with a rabbit polyclonal S-100 protein antibody (PA0900, 1:300; Leica Biosystems) and a rabbit polyclonal PGP 9.5 antibody (318A-15, 1:200; Cell Marque, CA) using a biotin-free polymeric horseradish peroxidase (HRP)-linker antibody conjugate system in a Bond-MAX automatic slide stainer (Leica Microsystems). The expression of antibodies in the samples was scored numerically by identifying peripheral nerve bundles in each specimen.

2.5. Statistical analysis

Statistical analysis was performed using SPSS (version 22.0; SPSS, Chicago, IL), and the level of significance was set at P < .05. Differences between the histological findings of the rotator cuff tear and control groups were evaluated using the Mann-Whitney U test. The correlation between histologic findings and clinical features was evaluated using the Spearman correlation.

3. Results

The average duration of symptoms was 10.2 ± 6.4 months, and the average preoperative VAS score was 2.9 ± 1.2 in the study group. The degrees of preoperative ROM in forward flexion, external rotation, and internal rotation were 143.8 ± 19.5°, 49.5 ± 23.1°, and −4.3 ± 4.2°, respectively. The average size of tear was 2.0 ± 0.9 cm (Table 1).

Routine H&E staining showed that the bursa consisted of flattened sacs of the synovial membrane supported by dense irregular connective tissue interposed with loose areolar tissue (Fig. 1). No mechanoreceptors were detected by the immunohistochemical staining. The control group had an average of 2–4 free nerve endings/low power field (LPF), whereas the average of 0–2 free nerve endings/LPF was found in the rotator cuff tear group (Fig. 2).

For histological findings, the number of neural elements per LPF in the rotator cuff tear group was significantly less than that in the control group in both immunohistochemical stainings (S-100: 0.5 ± 0.7 vs 2.8 ± 0.5, P < .01; PGP 9.5: 0.4 ± 0.7 vs 3.5 ± 0.6, P < .01) (Table 2).

In the correlation analysis, the number of neural elements in PGP 9.5, staining was negatively correlated with ROM in forward flexion and external rotation (P < .05, Table 3).

4. Discussion

Subacromial bursectomy is generally considered for several reasons:[14,15]: First, it is performed to remove the nociceptive nerve in SAB and origin of inflammatory cytokines.[2,4,5,16] Second, it is done to reduce the tension of rotator cuff tendon after repair surgery.[14] Third, it is done to obtain better visualization during arthroscopic rotator cuff repair.[13]

The key finding of this study is that the density of the neural elements in the SAB is significantly lower in the rotator cuff tears than in normal shoulders. Additionally, the number of neural elements showed a negative correlation with ROM in forward flexion and external rotation. In 1995, Vangsness et al.[17] first studied the neural elements in SAB using the shoulders of a normal cadaver and reported that there were diffuse free
endings in the SAB. In 1996, Sofier et al\cite{16} evaluated a normal cadaver shoulder using different staining techniques, namely S-100 staining for myelinated fibers and neurofilament protein staining for unmyelinated fibers to differentiate myelinated and unmyelinated fibers. They found rich myelinated and unmyelinated nerve fibers in the SAB.\cite{16} The result of our study are consistent with those of 2 previous studies. Furthermore, our results showed that there were abundant nerve fibers, including myelinated and unmyelinated nerves in a normal SAB.

Vangsness et al\cite{17} suggested that pain in shoulder disease may be caused by the stimulation of neural elements in the SAB. They supported the idea of performing bursectomy for pain relief. Additionally, Sofier et al\cite{16} agreed to remove the SAB and described that nociceptive information from free nerve endings in the SAB might be an aggravating factor for pain in rotator cuff pathology. Additionally, Ide et al\cite{1} reported free nerve endings and mechanoreceptors in the SAB. They assumed that the SAB is responsible not only for nociceptive nerves but also for proprioceptive nerves because there are various types of mechanoreceptors in the SAB.\cite{1} However, these conclusions are not sufficient to draw conclusions, as only normal shoulder joints, not the SAB in rotator cuff tear patients, were evaluated.\cite{1}

Therefore, our evaluation is valuable for evaluating the neural elements of SAB in patients with rotator cuff tears.

Yasuharu et al\cite{18} studied the neural elements of SAB in 57 patients with rotator cuff tears where they found numerous free nerve endings and mechanoreceptors. They also reported that the number of neural elements in the SAB apparently decreased with rotator cuff tears, and there was a negative correlation between the extent of tear and number of neural elements.\cite{18} Similarly, the number of neural elements in the rotator cuff tear group was lower than that in the control group in our study, indicating that the neural elements within the SAB are not the main components of the pain source in rotator cuff tears.

Neural elements are not the only sources of pain; inflammatory cytokines are another source of pain. According to previous studies, inflammatory cytokines, such as interleukin (IL) 1 and 6, tumor necrosis factor, cyclooxygenase 1 and 2, matrix metalloproteases, and substance P are increased in SAB with rotator cuff tears.\cite{2,4,5} Additionally, a study reported that pain in rotator cuff tears was strongly related to SAB inflammation.\cite{18}

The PGP 9.5 staining visualized all the free nerve endings. A previous study reported that some free nerve endings function as sensitive mechanoreceptors rather than nociceptors.\cite{19,20} Our study found that the PGP 9.5 staining has a negative correlation with ROM, indicating that the decreased neural elements may allow excessive joint ROM. This outcome was identical to that of a previous study, which considered the bursa to be a proprioceptive organ.\cite{1} The authors reported that the SAB was fixed at 2 sites, namely the coracoacromial arch and greater tuberosity.\cite{1} Around these sites, stretching or shrinkage of the SAB was induced by shoulder movements.\cite{1} Thus, they concluded that free nerve endings may detect changes in the SAB and function as a proprioceptive organ to prevent the shoulder from abnormal excessive movement.\cite{1} In the present study, the number of free nerve endings in the study group was shown to be decreased, resulting in excessive joint ROM due to lack of proprioceptive nerves.

Not only free nerve endings but also rotator cuff tears could affect ROM. However, previous studies have not proven a relationship between tear size and ROM. Hawkins et al\cite{21} reported no correlation between rotator cuff tear size and shoulder ROM. Another study reported the effect of tear size on ROM; however, there was only a significant difference in ROM between small and massive tears.\cite{22} There was no difference in ROM between

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**Table 1**

| Rotator cuff tear (n = 20) |
|----------------------------|
| **Sex (male/female)**      | 10/10 |
| **Age (yrs)**              | 61.9 ± 6.9 |
| **Symptom duration (mo)**  | 10.2 ± 6.4 |
| **VAS**                    | 2.9 ± 1.2 |
| **Range of motion**        |     |
| Flexion (degree)           | 143.8 ± 19.5 |
| External rotation (degree) | 49.5 ± 23.1 |
| Internal rotation (difference of spinal segment) | −4.3 ± 4.2 |
| Tear size (cm)             | 2.0 ± 0.9 |

VAS = visual analogue scale.

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Figure 1. Normal structure of subacromial bursa of control cadaveric specimen. (A) Villous synovial membrane (arrows) and 2 peripheral nerve bundles (★) in fibroadipose connective tissue (H & E, ×40), scale bar = 250 μm. (B) S-100 protein immunohistochemical stain expression in the Schwann cell (×200), scale bar = 50 μm. (C) PGP 9.5 immunohistochemical stain expression in the nerve axon (×200), scale bar = 50 μm.
small, medium, and large tears. The present study included patients with small, medium-sized, and large tears. Therefore, we assumed that the number of free nerve endings affected the ROM regardless of rotator cuff tear size.

The S-100 is a protein present in the cytoplasm of Schwann cells but not in fibroblasts or perineural cells. The S-100 staining tended to be negatively correlated with the duration of symptoms (correlation coefficient; –0.431, P = .058). In other words, the longer the duration of the rotator cuff tear states, the fewer the neural elements in the SAB.

We have suggested that degenerative changes in the bursa are major factors in reducing the neural elements in the SAB, based on the findings that chronic rotator cuff tears affect repetitive and abnormal movement of the shoulder and cause inflammation in SAB. According to previous studies, the extent of bursectomy did not affect the clinical outcomes of patients with rotator cuff tears, indicating that the remnants of the bursa did not affect the results. Other studies have reported that bursectomy resulted in no pain relief. Thus, rotator cuff repair is more clinically important than bursectomy.

Additionally, previous studies have emphasized the biological role of SAB, recommending avoiding unnecessary bursectomy in patients with rotator cuff tears. Several growth factors, such as vascular endothelial growth factor and transforming growth factor β, which are essential for tissue healing, have been detected in SAB. Another author reported that rotator cuff tears are covered with vascularized connective tissues and proliferating cells. They suggested that the SAB may play an important role in tendon reconstitution and remodeling. Decreased neural elements may affect this healing process. Therefore, in clinical situations, it is reasonable to perform arthroscopic rotator cuff repair prior to the destruction of the neural elements.

Mechanoreceptors encapsulate nerve endings. They act as a transducer that converts the mechanical energy of a physical formation into electrical energy of a nerve action potential. Wyke classified sensory nerve endings of the articular tissue into 4 types as follows: type I, Ruffini receptors; type II, lamellated (paciniform) receptors; type III, Golgi tendon organ receptors; and type IV receptors, which have free and unencapsulated terminals. According to Vangsness et al and Soiffer et al, no mechanoreceptors were found in the SAB. In contrast, Ide et al and Yasaharu et al described the presence of mechanoreceptors in the SAB. A recent study also reported the presence of mechanoreceptors in SAB. Nevertheless,

| Table 2 |
|---|---|
| **Histology of subacromial bursa** |  |
| | **S-100** | **PGP 9.5** |
| | Rotator cuff tear (n = 20) | Control (n = 4) | Rotator cuff tear (n = 20) | Control (n = 4) |
| Number of Neural element per LPF | 0.5 ± 0.7* | 2.8 ± 0.5* | 0.5 ± 0.7* | 3.5 ± 0.6* |

* P < .01 by Mann-Whitney U test.
LPF = low power field.

| Table 3 |
|---|---|
| **Correlation coefficient between histologic finding and clinical features** |  |
| | Symptom duration | VAS | Flexion | ER | IR | Tear size |
| S-100 | –0.431 | –0.286 | –0.146 | 0.005 | –0.296 | –0.191 |
| PGP 9.5 | 0.068 | –0.152 | –0.542* | –0.548* | –0.257 | 0.379 |

ER = external rotation, IR = internal rotation, VAS = visual analogue scale.
* P < .05 by Spearman correlation.
mechanoreceptors were absent in our study, and the probability of missing the mechanoreceptor was low because it is approximately 100 times larger than the nerve fibers. In this study, the preoperative VAS score measured just before the operation was 2.9 ± 1.2, which is relatively lower than that in other studies. In our institution, the average period between initial diagnosis and surgery was approximately 2–3 months, and the pain was initially controlled by NSAIDs during this period, while educating patients for shoulder rehabilitation, since preoperative rehabilitation can have a positive effect on postoperative results. Additionally, it is assumed that these preoperative preservation treatments may have affected the preoperative VAS score.

This study had several limitations. First, the sample size was relatively small, particularly for the control group. Second, the baseline characteristics were not controlled between the 2 groups because of the small sample size. Therefore, there may be confounding factors. Third, the SAB was evaluated only in the resected specimen rather than in the whole specimen. Fourth, histologic assessment is not quantitative, but semiquantitative. Fifth, postoperative changes in the neural elements were not evaluated. Future studies are needed to follow-up on the changes in neural elements in the SAB after surgery.

In conclusion, the neural elements of the SAB were decreased in rotator cuff tears. This study revealed that chronic rotator cuff tears may induce degeneration of neural elements in the SAB. Since the SAB provides proprioception for shoulder movement and healing of the rotator cuff tendons, in clinical situations, timely recognition, and treatments, such as rotator cuff repair are paramount to avoid irreversible nerve atrophy and functional deficits in the SAB.

Author contributions
Dong Rak Kwon contributed to the study of conception and design. Dong Rak Kwon, Chang-Hyuk Choi, Dae Gil Kwon, Hoon-Kyu Oh, and Jun Young Kim contributed to the interpretations of the study results, writing, and editing of this manuscript.

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