Histological and Elastic Evaluation of the Capsule in the Stiff Shoulder

Introduction:
Primary frozen shoulder is a common disease but its management is still difficult. The prevalence is estimated between 2 to 5% [1] and a criterion was initially described by Codmann in 1934 [2]. Both active and passive range of motion (ROM) is restricted and the pain is severe. The pathology of frozen shoulder still remains unclear. Inflammation and fibrosis were the predicted pathology but its etiology is still unknown. The capsule of the stiff shoulder was tightened from our experience while post-capsular release, however, was difficult to evaluate elasticity. Determination of expression levels of structural collagens is a key to understanding these elastic changes. The major structural collagens of the capsule are collagen types I and III, however, expression patterns of these collagens in the capsule of the stiff shoulder remain controversial. The purpose of this study was to elucidate the precise expression patterns of collagen types I and III in the capsule by quantifying real-time PCR, immunohistochemistry, and to calculate sound speed, which strongly correlate with tissue elasticity, with the capsule from the stiff shoulder (SS) and rotator cuff tear (RCT) as a control.

Materials and Methods:
The protocols of this study were approved by both institutional review boards of Funabashi Orthopaedic Clinic and Tohoku University. All patients consented before surgery. Biopsy material from the rotator interval (RI), middle glenohumeral ligament (MGHL) and inferior glenohumeral ligament (IGHL) were obtained through arthroscopic surgeries. Eleven samples from the stiff shoulder and 18 samples from the rotator cuff tear, with no limitation in ROM, were collected at each site.

Tissue Preparation: The samples were fixed with 4% paraformaldehyde in 0.1M phosphate-buffered saline and embedded in paraffin. The embedded tissue was cut into 5-μm thick slices. Hematoxylin and Eosin stain were prepared for cell counts.

Immunohistochemistry (IHC): The sections were deparaffinized and endogenous immunoglobulins were blocked. The slides were washed and incubated with a monoclonal mouse anti-human collagen type I antibody (F-56, Daiichi Fine Chemical Co., LTD, dilution 1:1600) or a monoclonal mouse anti-human collagen type III antibody (F-58, Daiichi Fine Chemical Co., LTD, dilution 1:1600). The slides were incubated with HRP conjugated goat anti-mouse antibody. The final detection step was carried out using DAB, 0.1 M imidazole, 0.03% hydrogen peroxidase as the chromogen.

Quantitative real-time PCR (qPCR): The other samples from the three sites were separately obtained and homogenized. The total RNA of the homogenate was purified and cDNA was synthesized. PCR efficiencies and relative expression levels of collagen types I and III as a function of EF1α1 were calculated.

Scanning Acoustic Microscope (SAM): Our new concept SAM consists of five parts: 1) ultrasonic transducer, 2) pulse generator, 3) digital oscilloscope with PC, 4) microcomputer board and 5) display unit. A single pulse ultrasound with 5 ns pulse width was emitted and received by the same transducer above the specimen. Distilled water was used as the coupling medium between the transducer and the specimen. The transducer was mounted on an X-Y stage with a microcomputer board that was driven by the computer installed in the digital oscilloscope through an RS-232C. Two-dimensional distributions of the ultrasonic intensity, sound speed and thickness of the 2.4 by 2.4 mm specimen area were visualized. The reflected waveform comprises two reflections at the surface and the interface between the tissue and the glass. The thickness and sound speed were calculated by Fourier-transforming the waveform. The sound speed of the capsule was calculated with a gray scale SAM image using commercially available image analysis software (PhotoShop CS3, Adobe Systems Inc., San Jose, CA) [3, 4].

Statistics: Differences between SS and RCT were compared at each time point by Mann–Whitney’s U test (qPCR) and by unpaired t-test (number of cells, and sound speed). Data were expressed as mean SD. A value of p<0.05 was accepted as statistically significant.

Results:
Number of Cells: The numbers of cells in the capsule (RI, MGHL, IGHL) were significantly increased in SS compared with those of RCT.

Figure 1: Number of cells in the IGHL. Hematoxylin and Eosin stain of RCT (A) and SS (B): SS. C: Average number of cells per unit. Scale bar = 100μm.

IHC and qPCR: Messenger RNA of both collagens was significantly increased in SS compared with RCT. Collagen type I expressed strong immunoreactivity in SS compared with RCT, however collagen type III was not changed.

Figure 2: qPCR and IHC of IGHL. A: quantitative PCR of collagen types I and III, B and D: RCT, C and E: SS. IHC of collagen type I (B and C) and collagen type III (D and E). Scale bar = 100μm.

SAM: Gradation color images of SAM indicated high sound speed in SS compared with that in RCT. Average sound speed of the capsule (RI, MGHL, IGHL) was significantly increased in SS compared with that in RCT.

Figure 3: Sound speed changes of the IGHL. Gradation color images of RCT (A) and SS (B). C: Average sound speed.

Discussion: Increased numbers of cells and expression of collagen types I and III suggested proliferative changes of the capsule in SS. However, it was still unclear how these collagens affected increased sound speed of the capsule in SS. Further study was needed to clarify the changes of the capsule in SS.


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