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Synovial fluid biomarkers: association with chronic rotator cuff tear severity and pain

Chien-An Shih, MD[a], Kuo-Chen Wu, MD[b], Chung-Jung Shao, MD[c], Tai-Chang Chern, MD[d], Wei-Ren Su, MD[a,e,f,g], Po-Ting Wu, MD, PHD[a,e,f,g,h,*], I-Ming Jou, MD, PHD[f,i,]**

[a]*Department of Orthopedics, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, Taiwan*
[b]*Department of Orthopedics, Kuo's General Hospital, Tainan, Taiwan*
[c]*KSP Orthopedics Clinic, Taichung, Taiwan*
[d]*Chern Tai-Chang's Orthopedics Clinic, Ping-Tong, Taiwan*
[e]*Department of Orthopedics, College of Medicine, National Cheng Kung University, Tainan, Taiwan*
[f]*Medical Device Innovation Center, National Cheng Kung University, Tainan, Taiwan*
[g]*Medical Device R & D Core Laboratory, National Cheng Kung University Hospital, Tainan, Taiwan*
[h]*Department of Biomedical Engineering, National Cheng Kung University, Tainan, Taiwan*
[i]*Department of Orthopedics, E-Da Hospital, Kaohsiung, Taiwan*

Background: We tested the hypothesis that biomarkers in the synovial fluid of the glenohumeral (shoulder) joint are correlated with visual analog scale (VAS) scores, functional scores, and ultrasound findings of chronic rotator cuff tear (RCT) severity.
Methods: We measured biomarkers in shoulder joint synovial fluid of 42 patients with partial-thickness (21), nonmassive full-thickness (10), and massive full-thickness (11) RCTs. Pain duration, tear severity, and VAS and functional scores were compared with interleukin (IL) 1β, IL-6, matrix metalloproteinase (MMP) 1, and MMP-13 levels.
Results: Both MMP-1 and MMP-13 levels were significantly highest in the massive full-thickness group. MMP-13 levels were significantly different between groups, but proinflammatory cytokine IL-1β and IL-6 levels were not. However, IL-1β levels were significantly positively correlated with VAS ($r = 0.66$; $P < .01$) and functional scores ($r = 0.61$; $P < .01$), but IL-6, MMP-1, and MMP-13 levels were not.
Conclusions: IL-1β levels in shoulder synovial fluid correlated positively with shoulder pain and functional scores in patients with chronic RCTs. Both MMP-1 and MMP-13 levels were altered and increased with cuff tear severity.

The Human Experiment and Ethics Committee of National Cheng Kung University Hospital approved this study: No. HR-95-85. All patients signed a written informed consent form before participating in the study.

*Reprint requests: Po-Ting Wu, MD, PhD, Department of Orthopedics, College of Medicine, National Cheng Kung University, 1 University Road, Tainan 701, Taiwan.
E-mail address: anotherme500@gmail.com (P.T. Wu).
**Reprint requests: I-Ming Jou, MD, PhD, Department of Orthopedics, E-Da Hospital, 1, Yi-Da Road, Jiao-Su Village, Yen-Chao District, Kaohsiung City 824, Taiwan.
E-mail address: ed109325@edah.org.tw (I.M. Jou).

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Chronic rotator cuff tears (RCTs) are a common degenerative occurrence; they are associated with pain, disability, and a decrease in quality of life.[15,21] Clinically, associated extrinsic anatomic factors do not always reflect the severity of the RCT or the glenohumeral (shoulder) pain.[11,18,29] Rather, increasing evidence shows that intrinsic factors might be important in RCT progression, shoulder function, and shoulder pain.[5,14,15,37,46] Interleukin (IL) 1β and IL-6 are considered the upstream mediators of inflammation that contribute to tendinopathy, and they might cause rotator cuff tendinopathy.[42,45] Few studies[15,26,46] have examined the association between IL-1β and IL-6 and shoulder pain in chronic RCTs. Two studies[15,38] showed an inconsistent correlation between the level of inflammatory cytokine IL-1β in the subacromial bursa and the severity of pain in patients with RCTs.

Matrix metalloproteinases (MMPs) are also associated with inflammation.[22] They can degrade extracellular matrix proteins and activate cytokines and chemokines.[22] In MMP families, MMP-1 and MMP-13 are major collagenases that are able to cleave nearly all subtypes of collagen.[10] However, their expression levels vary in patients with RCTs.[22,47] The functions of MMPs in RCTs are still unclear.[21,41]

Shoulder synovial fluid is a pooled environment of biomarkers from surrounding shoulder tissue.[39] There are no studies analyzing the relationship between biomarkers in shoulder joint synovial fluid and severity of shoulder pain in chronic RCTs. In this study, we collected the shoulder joint synovial fluid of patients with chronic RCTs and compared the proinflammatory cytokine (IL-1β, IL-6) and collagenase (MMP-1 and MMP-13) levels with their visual analog scale (VAS) scores, RCT severity, radiographic Hamada grade, and Western Ontario Rotator Cuff (WORC) index. We hypothesized that in patients with chronic RCTs, a higher synovial fluid IL-1β level is associated with more severe pain and poorer functional scores and that the synovial fluid MMP-1 and MMP-13 levels indicate severity of the RCT.

Materials and methods

Patient groups and clinical assessments

This was an analytic, level IV, case-control study. We recruited consecutive patients with symptomatic RCTs between June 2009 and April 2010 and referred all patients to our outpatient clinics for an intra-articular injection. We compared their IL-1β, IL-6, MMP-1, and MMP-13 levels in synovial fluid with RCT severity, WORC index, radiographic Hamada grade, and VAS score. Inclusion criteria were a chronic rotator cuff lesion (duration >3 months) and conservative medical treatment with physical therapy for ≥3 months. Exclusion criteria were concomitant RCT arthropathy, metabolic disease, connective tissue disease, malignant disease, instability, previous shoulder trauma, prior shoulder surgery, loss of shoulder motion (passive elevation <110°), and history of previous shoulder intra-articular injection.[15] We also excluded patients without data for IL-1β, IL-6, MMP-1, and MMP-13 levels. Shoulder ultrasound (US) scans and routine shoulder anteroposterior and lateral radiographs were obtained. A magnetic resonance imaging (MRI) examination was performed when the US diagnosis was not definitive.

We evaluated the degree of shoulder pain using a VAS[15] graded with scores from 0 to 10: mild pain, 1-3; moderate pain, 4-6; and severe pain, 7-10. We evaluated shoulder function using the WORC total score (range, 0-2100); a higher raw score indicated worse function.[25]

US examination, Hamada radiographic classification, and synovial fluid biomarker analysis

Two experienced operators performed the US shoulder examinations. RCT severity was classified as partial-thickness tear (PTT), nonmassive full-thickness tear (NM-FTT), and massive full-thickness tear (M-FTT). A PTT is defined as an articular or bursal surface lucent patch in the tendon with remaining fibers to the greater tuberosity.[19] An FTT is defined as a full-thickness lucent patch going through the tendon.[19,30] Because of variations in measurement techniques and size of the patients, FTTs that involved only 1 tendon were defined as nonmassive; those that involved 2 or more entire tendons were defined as massive, which indicates more severe tears.[13,44] An MRI examination was performed when US diagnosis was uncertain.

We used the Hamada classification[16] to evaluate the radiologic grades of massive RCTs. Severity was based on radiographic evidence (Fujifilm, Tokyo, Japan): grade 1, acromiohumeral interval ≥6 mm; grade 2, acromiohumeral interval ≤5 mm; grade 3, grade 2 plus acromion acetabulization; grade 4, grade 3 plus glenohumeral joint narrowing; and grade 5, humeral head collapse.

Shoulder joint synovial fluid was collected by US-guided aspiration.[12,43] After the fluid had been centrifuged and the debris removed, the supernatants were stored at −80°C until Western blotting or an enzyme-linked immunosorbent assay (ELISA) was done. IL-1β and IL-6 levels were calculated using ELISA kits (BMS224/2 and BMS213/2; eBioscience, San Diego, CA, USA). Optical density was measured using spectrophotometry. MMP-1 and MMP-13 were analyzed; total protein concentration was estimated using a Bradford protein assay.[6] The synovial fluid was then incubated with the primary antibodies of MMP-1 and MMP-13 (Abcam Plc, Cambridge, UK), visualized (Fujifilm), digitized, and analyzed (Image-Pro Plus 4.5.1; Media Cybernetics, Rockville, MD, USA).