The severity of the long head biceps tendinopathy in patients with chronic rotator cuff tears: macroscopic versus microscopic results

Po-Ting Wu, MDa,b, I-Ming Jou, MD, PhDb, Cheng-Chang Yang, MSCc, Chii-Jeng Lin, MD, PhDb, Chyun-Yu Yang, MD, PhDb, Fong-Chin Su, PhDa, Wei-Ren Su, MD, MSCb,*

aInstitute of Biomedical Engineering, National Cheng Kung University, Tainan, Taiwan
bDepartment of Orthopedics, National Cheng Kung University Hospital, Tainan, Taiwan
cInstitute of Basic Medical Sciences, National Cheng Kung University, Tainan, Taiwan

Background: This study investigated the histopathology of the long head of biceps (LHB) tendon and correlated the findings with the macroscopic appearances of the LHB and the size of rotator cuff tears (RCTs) in patients with chronic RCTs.

Methods: We compared biopsy specimens from LHBs in 34 patients with chronic RCTs and grossly normal LHBs in 8 patients undergoing shoulder hemiarthroplasty (controls). Duration of preoperative symptoms, the severity of RCTs, and macroscopic appearance of LHBs were recorded, classified, and compared with the histologic grading and apoptosis index of terminal deoxynucleotide transferase-mediated biotin-deoxy uridine triphosphate nick-end labeling (TUNEL) assays of LHBs.

Results: In the RCT group, there were 8 partial-thickness tears with 5 macroscopic LHB lesions, 12 full-thickness tears with 8 macroscopic LHB lesions, and 14 massive tears with 13 macroscopic LHB lesions. There were 6 LHB subluxations. However, the macroscopic grading and the symptom duration were not correlated with the severity of the histology. In patients with massive tears, no matter what the macroscopic appearance of the LHB, the proportion of end-stage (grade 4) histologic LHB tendinopathy significantly increased (85.7%, P < .05) compared with patients with other types of RCTs. There was a consistently high incidence of advanced LHB histology (grade 3 or higher) in each classification of RCTs (75.0%-100.0%). The 8 patients in the control group showed milder histopathology (grade 1 or 2). The apoptosis index significantly increased as the tendinopathy progressed (P < .05).

Conclusions: The macroscopic pathology of LHB may not fully reflect the severity of tendinopathy, and the coexisting size of RCTs plays a role in the severity of LHB tendinopathy.

Level of evidence: Basic Science Study, Histology.

Keywords: Long head of biceps; tendinopathy; rotator cuff tear; tenotomy/tenodesis; apoptosis; shoulder pain

This study was approved by the Institutional Review Board of National Cheng Kung University Hospital (Protocol No./IRB No.: NCKUH-10002009/ER-99-260).

*Reprint requests: Wei-Ren Su, MD, MSc, Department of Orthopedics, National Cheng Kung University Hospital, 138 Sheng-Li Rd, Tainan 704, Taiwan.
E-mail address: suwr@ms28.hinet.net (W.-R. Su).
Shoulder pain is common in chronic rotator cuff tears (RCTs) and is possibly caused by long head of biceps (LHB) tendinopathy.\textsuperscript{4,5,23,30,32} Clinically, damage to the LHB is often seen in conjunction with RCTs but rarely in isolation.\textsuperscript{9,13} Many studies\textsuperscript{9,14,25,29} have shown that the LHB has a high incidence of macroscopically pathologic lesions in an RCT, especially in a massive tear (MT). How to manage concomitant LHB tendinopathy in procedures dealing with RCTs is still a challenge. Removing a potentially advanced pathologic LHB tendon when doing a rotator cuff repair or debridement may be acceptable because it may be mechanically deteriorated and, therefore, prone to rupture.

In a RCT repair, the current indications for an LHB tenotomy or tenodesis are based primarily on the morphology of the biceps.\textsuperscript{26,27} A discrepancy between the macroscopic and microscopic findings in LHB lesions may exist, and the relationship between chronic RCTs and the histopathologic changes of concomitant LHB is important and helpful in decision making. However, there is still no such study in the literature.

The purpose of this study was to investigate the histopathology and apoptosis expression of the LHB tendon in a prospective case-control study of patients with chronic RCTs and correlate the findings with the macroscopic appearances of the LHB and the size of the RCTs. We hypothesized that the macroscopic appearance of the LHB is not consistent with its histologic severity and that LHB accompanying massive RCTs presents a higher incidence of histologically end-stage tendinopathy compared with those accompanying other sizes of rotator cuff lesions.

**Materials and methods**

**Patient groups**

The study recruited 34 consecutive patients (14 men and 20 women; mean age at surgery, 56.9 years; range, 34-73 years) undergoing arthroscopic treatment for an RCT with or without other ipsilateral shoulder lesions at our university hospital between January and December 2011. The inclusion criterion was a chronic rotator cuff lesion (duration ≥3 months).\textsuperscript{8} The exclusion criteria were concomitant frozen shoulder syndrome, inflammatory arthritis, connective tissue disease, and age <18 years. The demographic data of patients were recorded, and the patients were assigned to 1 of 2 groups by the duration of their preoperative symptoms: ≤6 months and >6 months.\textsuperscript{1} The mean ± standard deviation of symptom duration was 9.6 ± 5.0 months (range, 3-24 months). Fourteen patients (41.2%) underwent surgery ≤6 months after the onset of symptoms.

All patients were given a preoperative physical examination and a magnetic resonance (MR) image (MRI) scan or MR arthrography to evaluate the rotator cuff, the LHB, and the labrum lesion, if there was one. The final diagnosis was confirmed using the arthroscopic findings and then recorded. During surgery, the rotator cuff tendon lesion was identified and classified as a partial-thickness tear, a full-thickness tear, or a massive tear. Because of variations in the techniques of measurement and the sizes of the patients, complete RCTs that involved 2 or more entire tendons were defined as massive tears\textsuperscript{13} to represent more severe tears.

A simplified classification modified from a prior study\textsuperscript{9} was created to describe the macroscopic pathologic lesion of the LHB. Type 0 was the macroscopically normal tendon. The type I lesion was defined as tendinitis of the tendon, type II was fibrillation or delamination of the tendon, type III was a tendon tear of less than 50% of the tendon width, type IV was tendon tear of more than 50% of the tendon width (Fig. 1, A-D). The location of the LHB was classified as in the bicipital groove, subluxation, or dislocation.

The control group comprised 8 consecutive patients (2 men and 6 women) who were a mean age of 64.0 years (range, 56-72 years) who underwent shoulder hemiarthroplasty for proximal humeral fractures with an intact rotator cuff and a macroscopically normal (type 0) LHB inspected during surgery.\textsuperscript{17} All patients provided written, signed, informed consent beforehand for all subsequent procedures.

**Surgical procedure and biopsy**

At the beginning of all procedures, the LHB was first evaluated using a “dry” scope examination with no pump pressure. The intertubercular groove portion of the LHB had to be retracted into the joint for evaluation. The rotator cuff lesions were treated as follows: if the tear thickness was less than 50%, the treatment was debridement with or without acromioplasty. If the tear thickness was more than 50% or was complete, the treatment was arthroscopic repair. Treatment for types I and II LHB lesions was only debridement. For type III lesions, if there was coexisting LHB subluxation or dislocation, or a massive RCT, resection or tenodesis was done; otherwise, only debridement was done. The treatment for all type IV lesions was tenodesis or resection.

The LHB tendons were arthroscopically harvested from the macroscopic lesion site (Fig. 2). For type 0 tendons, the specimens were harvested from the lateral border of the tendon 1 to 1.5 cm distal to the superior labrum, the location of the common tear site of the LHB.

**Histologic grading and detecting apoptosis**

The specimens were fixed in fresh 4% paraformaldehyde for 16 to 24 hours at 4°C, then subsequently dehydrated, embedded in paraffin, and longitudinally sectioned. Sequential 5-μm sections were stained with hematoxylin and eosin and examined under a light microscope. Light and polarization microscopy was used to evaluate tendons for changes in tenocyte morphology and collagen bundle characteristics. As detailed in previous studies,\textsuperscript{12} we used a semiquantitative method to score each factor on a 4-point scale. According to the sum of scores, the tendinopathy was graded as 0 to 4 (0, ≤2, 3, 4, ≥5 points; Fig. 1, E-H). Grades 3 and 4 were defined as advanced tendinopathy; furthermore, grade 4 was considered end-stage tendinopathy. The histologic grading was assessed by 2 observers unaware of the clinical and arthroscopic findings of patients.

A terminal deoxynucleotidyl transferase-mediated biotin-deoxy uridine triphosphate nick-end labeling (TUNEL) assay was used to identify apoptotic cells by labeling nuclear DNA fragments (In Situ Cell Death Detection Kit, AP 1684817; Roche Diagnostics...
GmbH, Mannheim, Germany; Fig. 1, I-L). The TUNEL-positive control was a specimen incubated with DNase I dissolved in 1 mM MgSO₄ containing triethanolamine-buffered saline before the following procedure, and the negative control was a specimen without the terminal deoxynucleotidyl transferase enzyme. The specimens were stained with methyl green and mounted with p-xylene-bis(N-pyridinium bromide) Permount (14208-10-7; Sigma-Aldrich, Castle Hill, NSW, Australia).

Positive cells were counted in a high-power field (original magnification /C 2 100) with a distinct morphologic appearance. This was repeated in 5 randomly selected high-power fields. The average frequency of positive cells was expressed as the apoptotic index, a percentage relative to the number of cells counted. In addition, cell density (cells/mm²) was also calculated as the number of cells in the randomly selected high-power fields divided by the area of analysis.

**Statistical analysis**

Differences in patient age, apoptotic index, and cell density between histologic grades were analyzed using a Kruskal-Wallis test because of the relatively small number of patients. The intergrade difference was analyzed using a post hoc Mann-Whitney U test. The correlations between the severity of the tendinopathy and the macroscopic appearance and between the severity of the tendinopathy and the symptom duration were analyzed using Pearson's correlation test with Cramer's V coefficient and a post hoc test, if necessary. The proportion of the tendinopathy grades in each RCT classification was analyzed using a \( \chi^2 \) test with Cramer's V coefficient and a post hoc test if necessary. Significance was set at \( P < .05 \). The interobserver agreement of histopathologic grades was evaluated using the \( \kappa \) value. Assessment discrepancies were resolved by consensus. Data were analyzed using SPSS 16.0 software (SPSS Inc, Chicago, IL, USA).

**Results**

**Macroscopic and microscopic appearance of LHB**

In 34 patients with rotator cuff lesions, the duration of preoperative symptoms was not correlated with the severity of the histology (\( P = .529 \)). The overall incidence of macroscopic LHB lesion (type I or higher) was 76.5% (26 of 34; Table 1). There were 6 LHB subluxations distributed in 2 macroscopic type II lesions, 1 type III lesion, and 3 type IV lesions. There were no LHB dislocations. The overall incidences of macroscopic LHB lesions
were 62.5% (5 of 8) for incomplete rotator cuff lesions, 66.7% (8 of 12) for complete lesions, and 92.9% (13 of 14) for massive lesions (Table I). The incidence of LHB macroscopic tendinopathy was higher, but nonsignificant, in massive RCTs. In the control group, 6 patients had histologically grade 1 LHB tendinopathy and 2 patients had grade 2. However, in patients with chronic RCTs, the macroscopic grading was not correlated with the microscopic severity of tendinopathy (P = .199; Table II). In our patients, macroscopic type III and IV lesions both had a high proportion of grade 4 histology (11 of 13). The histology of the macroscopically normal LHB in patients with chronic RCTs showed a 75% incidence (6 of 8) of advanced tendinopathy and none with normal histology. For other types of macroscopic lesions, the histology results varied, but none were normal.

In patients with massive rotator cuff lesions, no matter what the macroscopic appearance of the LHB was, the proportion of end-stage LHB tendinopathy was significantly higher (85.7%, P = 0.013; Table III) than in patients with incomplete or complete RCTs. In all patients with chronic rotator cuff lesions, the proportion of advanced microscopic tendinopathy (grade 3 + 4) of LHB was high (75.0%-100.0%) but not significantly different (P = .174; Table III) in each classification of rotator cuff lesions. Interobserver agreement on histopathologic grades was excellent (r = 0.91).

**Apoptosis and cell density in LHB tendinopathy**

The apoptotic index significantly increased as the tendinopathy progressed (Fig. 3, A). The cell density increased, peaked at grade 2 tendinopathy, and then dropped gradually as the tendinopathy progressed (Fig. 3, B). The distribution of the grades of LHB tendinopathy by age was not significantly different (Fig. 3, C).

**Discussion**

This is the first study that uses histopathology and immunohistochemistry to clarify the relationship between the severity of LHB tendinopathy and the extent of RCTs and the correlation between the macroscopic appearance and severity of LHB tendinopathy in patients with chronic tears. The incidence of macroscopic LHB tendinopathy was higher, but not significantly, in patients with massive RCTs. The macroscopic grading and the duration of preoperative symptoms, however, were not correlated with the histologic severity of tendinopathy. Regardless of the macroscopic appearance of LHB, there was a significantly higher proportion of grade 4 LHB tendinopathy in patients with massive RCTs and a consistently high ratio of advanced tendinopathy (grade 3 or higher) in all patients with chronic RCTs. As the tendinopathy progressed, the apoptosis ratio increased and the cell density decreased.

The symptomatic significance of LHB has been increasingly recognized as an important source of persistent shoulder pain. The literature suggests that there may be a causal relationship between LHB lesions and rotator cuff tendinopathy. The function of the LHB still remains controversial: it has been called an anterior stabilizer, a posterior stabilizer, and a head depressor. However, the current consensus is that once the RCT occurs, subacromial impingement or increased abnormal mechanical loading on the LHB will cause progressive deterioration. Therefore, LHB tendinopathy has been regarded as the result of an ongoing subacromial impingement syndrome and rotator cuff tendinopathy, as was shown in an animal study. Variable incidences (29.0%-85.7%) of macroscopic LHB lesions in RCTs have been reported. In our study, there was a high incidence (26 of 34 [76.5%]) of macroscopic pathologic change of the LHB in chronic RCTs, especially in massive RCTs (13 of 14 [92.9%]). Our finding of a remarkably high incidence of LHB lesions in patients with chronic massive RCTs agrees with prior results (88.9%-100%).

Because of the high incidence of LHB lesions in patients with chronic RCTs, the evaluation and treatment of these lesions should be emphasized. Current treatment suggestions are based primarily on the macroscopic findings of LHB lesions. Indications for biceps tenodesis/tenotomy are tears involving >25% to 50% of the tendon, medial subluxation of the biceps tendon, and combined subscapularis tears and biceps subluxation. Nevertheless, the indication for these procedures is equivocal in patients with arthroscopic evidence of <30% to 50% of intra-articular LHB fraying without instability. To date, no published prospective randomized controlled study has
addressed the question of whether to treat LHB in addition to rotator cuff repair. However, we found that the macroscopic appearance does not always reflect the histologic grade. Almost all macroscopic type IV lesions represent a grade 4 histology of tendinopathy. In contrast, macroscopically normal LHB do not represent milder tendinopathy and may just as easily reflect various degrees of histologic tendinopathy instead. According to the classification of rotator cuff lesions, no matter what the macroscopic appearance of the LHB was, we found a significantly higher proportion of microscopically end-stage (grade 4) LHB tendinopathy in patients with massive RCTs. Our results showed that the macroscopic findings of LHB may not be the only foundation for treatment during the arthroscopic rotator cuff repair and that the coexisting extent of rotator cuff lesions should be taken into consideration. This is one possible reason that some patients still had persistent anterior shoulder pain with a normal LHB tendon at the time of the rotator cuff repair for massive tears or that patients experienced pain relief from the LHB tenotomy/tenodesis in irreparable RCTs. However, the reasons leading to the discrepancy between macroscopic and microscopic results remain unclear and require additional studies.

Apoptosis is a normal physiologic process that is usually underway in 3% to 10% of healthy adult tissue. Inflammation, mechanical overuse and injury, and subsequent oxidative stress are among the factors that may lead to increased apoptosis in rotator cuff tendinopathy. As with rotator cuff tendinopathy, LHB tendinopathy caused by a mechanical injury because of an ongoing subacromial impingement and mechanical overloading after a RCT may lead to increased apoptosis.

Apoptotic cell death has been postulated to be a primary cause of tendinopathy rather than a secondary effect of degeneration, and its expression is in direct proportion to the severity of the tendinopathy. Excess apoptotic leads to decreased cell density, which leads to an impaired homeostasis of the collagen matrix and a reduced healing response to repeated microinjuries. Therefore, overexpressed apoptosis is a hallmark in degenerating human tendon tissue.

We found a significantly incremental increase in the apoptotic index with a worsening histologic grade. Cell density decreased significantly in advanced tendinopathy.

<table>
<thead>
<tr>
<th>Variable</th>
<th>LHB macroscopic type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Etiology</td>
<td>Control: 8 PTTs: 2</td>
</tr>
<tr>
<td></td>
<td>PTTs: 3 FTTs: 2</td>
</tr>
<tr>
<td></td>
<td>FTT + SLAP type II: 1</td>
</tr>
<tr>
<td></td>
<td>MTs: 1</td>
</tr>
<tr>
<td></td>
<td>Total No. 16 8 5 5 8</td>
</tr>
</tbody>
</table>

**Table II** The number of long head of biceps (LHB) macroscopic types in the histology grades 0 to 4

<table>
<thead>
<tr>
<th>LHB histology</th>
<th>LHB macroscopic type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0</td>
<td>0</td>
</tr>
<tr>
<td>Grade 1</td>
<td>6 (control)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>2 + 2 (control)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>4</td>
</tr>
<tr>
<td>Grade 4</td>
<td>2</td>
</tr>
<tr>
<td>Total No.</td>
<td>16</td>
</tr>
</tbody>
</table>

**Table III** The etiology of rotator cuff lesions in histology grades 0 to 4 with concomitant long head of biceps (LHB) tendinopathy

<table>
<thead>
<tr>
<th>LHB histology</th>
<th>Etiology of rotator cuff lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0</td>
<td>PTTs: 0  FTTs: 0  MTs: 0  Control group</td>
</tr>
<tr>
<td>Grade 1</td>
<td>PTTs: 0  FTTs: 0  MTs: 0  Control group</td>
</tr>
<tr>
<td>Grade 2</td>
<td>PTTs: 2  FTTs: 0  MTs: 0  Control group</td>
</tr>
<tr>
<td>Grade 3</td>
<td>PTTs: 0  FTTs: 0  MTs: 0  Control group</td>
</tr>
<tr>
<td>Grade 4</td>
<td>PTTs: 0  FTTs: 0  MTs: 0  Control group</td>
</tr>
<tr>
<td>Total No.</td>
<td>8 12 14 8</td>
</tr>
</tbody>
</table>

* Two patients were diagnosed with LHB subluxation.
* One patient was diagnosed with LHB subluxation.
* One patient was diagnosed with LHB subluxation.
* Two patients were diagnosed with LHB subluxation.
Our finding that increased apoptotic cell death and decreased cell density are features of LHB tendinopathy are compatible with those in tendinopathy of the rotator cuff, patellar tendon, and extensor carpi radialis brevis.3,10,18,22,33 In the control group, all patients showed grade 1 or 2 tendinopathy with a mildly increased apoptotic index. To our knowledge, this is the first report of apoptotic cell death in the LHB tendon.

On the basis of our histologic and immunohistochemical findings, most LHBs in chronic RCTs showed advanced tendinopathy. In patients with chronic massive RCTs, the incidence of end-stage LHB tendinopathy was remarkably higher whatever their macroscopic appearances were. End-stage tendinopathy suggests that decreased cell density diminishes the synthesis of collagen and the capacity for healing after injury. This may lead to a weaker tendon, susceptibility to further injury, possible pain, and possibly, an eventual rupture. Our findings reflect not only the high incidence of end-stage biceps tendinopathy but also increased apoptotic cell death and decreased cell density in chronic massive RCTs.

Our study has some limitations: First, the sample size was small. However, the high incidence of microscopically end-stage LHB tendinopathy in the massive rotator cuff category was still significant.

Second, the age difference in individuals possibly affects the apoptotic index expression. However, the ages in different histologic grades were not significantly different in our study.

Third, we did not correlate the physical examination results with the histologic findings. Previous studies reported that biceps-related tests fail to correlate with the intraoperative biceps pathology because the LHB symptoms can be diminished by symptoms resulting from concomitant rotator cuff lesions.2,16 Otherwise, the current indication of LHB tenotomy or tenodesis is based primarily on the macroscopic appearance of LHB. Therefore, we correlated the arthroscopic findings only with the histological results.

Fourth, the TUNEL assay also detects necrotic cells and autolytic cell death, although it has been used as the method of choice for detecting apoptosis in situ.20
Finally, we did not evaluate the effects of the discrepancy between macroscopic and microscopic results on clinical outcomes because our management of LHB still stood on current macroscopic indications. Additional studies to confirm the discrepancy and the clinical effects are necessary. However, our findings may give rise to additional research on therapeutic strategies on LHB tendinopathy in chronic RCTs.

Conclusion

The macroscopic pathology of LHB may not fully reflect the severity of tendinopathy, and the size of RCTs plays a role in the severity of LHB tendinopathy.

Acknowledgments

The authors are honored to acknowledge the National Science Council of TAIWAN for funding this work (NCS 102-2314-B-006-022), and grateful to Wei-Ting Chang, Shing-Yun Chang, and Yu-Ying Chen for their excellent assistance in data collection and preparation.

Disclaimer

The authors, their immediate families, and any research foundations with which they are affiliated have not received any financial payments or other benefits from any commercial entity related to the subject of this article.

Supplementary data

Supplementary data, including detailed demographic data for all patients in this study, can be found online at http://dx.doi.org/10.1016/j.jse.2013.11.013.

References


