QUANTIFICATION OF CELLULARITY AND LIPOID DEGENERATION TO ASSESS LOCALIZED DEGENERATION IN ROTATOR CUFF TENDONS

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INTRODUCTION
Degenerative rotator cuff tears are a common problem, affecting greater than 30% of the general population [1]. Rotator cuff tears most commonly occur in the supraspinatus tendon near the insertion site [2]. There is a high failure rate of rotator cuff tear repairs, and this failure typically occurs at the tendon-suture interface [3]. By determining localized differences in rotator cuff tendon degeneration, surgical techniques can be improved. Past studies have evaluated localized tendon degeneration, but these studies only focused on one location in the tendon, such as the medial edge of a chronic tear [2]. Sano et al. discovered a relationship between decreased tendon mechanical properties and increased tendon degeneration at the insertion of the supraspinatus tendon [2]. Therefore, the objective of this study is to evaluate localized tendon degeneration in multiple locations by quantifying cellularity and lipid degeneration, two characteristics of tendon degeneration.

OBJECTIVE
The objective of this project is to determine histological differences in rotator cuff tendons based on cell number and lipid degeneration by comparing tendons with and without rotator cuff tears. The goal was to validate the results of a prior study that was conducted, which evaluated cell number and lipid degeneration using a semi-quantitative grading scale, and to provide quantitative, location-based degeneration information.

HYPOTHESIS
It was hypothesized that there would be more lipid degeneration in the supraspinatus than the infraspinatus, since most rotator cuff tears occur in the supraspinatus tendon. It was also hypothesized that there would be more cellularity in tendons with chronic tears than in intact tendons. The success criteria for this project include finding quantitative degeneration information based on the anterior and posterior regions of the insertion, mid-substance, and junction regions within the supraspinatus and the infraspinatus tendons.

METHOD
Eight fresh-frozen cadaveric shoulders were used in this study. Four shoulders were intact (55 ± 12 years) and four had small tears in the supraspinatus tendon (70 ± 9 years). Tissue biopsy samples were taken from the anterior and posterior sides of the insertion, mid-substance, and myotendinous junction regions on the supraspinatus tendon. Samples were also taken from the infraspinatus, in the same regions as the supraspinatus.

For specimens with a chronic tear, samples were also taken from the medial edge of the tendon tear. The samples were fixed in a 10% buffered formalin solution for at least three days. Then samples were sectioned at a thickness of 5 µm and cut longitudinally, stained with hematoxylin and eosin (H&E) to visualize tendon morphology, and imaged using a light microscope with a 20x objective lens across the full tendon thickness. All images were evaluated based on cellularity and lipid degeneration [Figure 1]. To quantify cellularity, the number of nuclei in each sample was counted using ImageJ software (Fiji, NIH). For this method, an intra-observer repeatability of 0.905 and an inter-observer repeatability of 0.725 were calculated. For lipid degeneration analysis, ImageJ software was utilized to determine the percent area of lipid degeneration per sample. An intra-observer repeatability of 0.992 and an inter-observer repeatability of 0.986 for this method was calculated. A two-way ANOVA and a post-hoc test was conducted to compare degeneration based on location and whether the specimen had a tear, with a significance of p<0.05. Degeneration comparing the supraspinatus vs. infraspinatus and intact vs. chronic tear were performed using a paired t-test.

Figure 1. Histological images of rotator cuff tendons. A) Intact infraspinatus tendon mid-substance with healthy cells and no lipid degeneration. B) Chronic tear supraspinatus tendon exhibiting increased cellularity. C) Chronic tear infraspinatus tendon at myotendinous junction showing lipid degeneration.

RESULTS
While tendons showed varying degrees of degeneration, there was no statistically significant difference between the supraspinatus and infraspinatus based on lipid degeneration. It was also found that there was no statistical significance between intact and chronic tears based on cellularity. However,
a significant interaction effect existed between location and the presence of a tear for cellularity (p=0.016). The tendon mid-substance showed significantly more cellularity in specimens with chronic tears when compared to intact tendons (p=0.024) [Figure 2].

![Figure 2](image)

**Figure 2.** Cellularity for each sample location based on tendon state: intact and torn. (mean +/- S.D.)

Tendons with chronic tears showed significantly more lipoid degeneration in the myotendinous junction when compared to intact tendons (p=0.006) [Figure 3].

![Figure 3](image)

**Figure 3.** Lipoid degeneration for each sample location for intact and torn tendons. (mean +/- S.D.)

For both cellularity and lipoid degeneration, it was found that there was a positive correlation between the objective scores (from this study) and the subjective scores (from the semi-quantitative study) between the grading variables for the insertion, mid-substance, and junction regions for p<0.05. For cellularity, the Spearman’s non-parametric statistical test calculated a correlation coefficient of 0.64, with a p-value of 1x10^-5. For lipoid degeneration, the Spearman’s non-parametric statistical test calculated a correlation coefficient of 0.87, with a p-value of essentially 0. Therefore, this study validated the results from the semi-quantitative study.

**DISCUSSION**

The results of this study showed that tissue degeneration is spread throughout the tendon, and is not localized to one specific location. As lipoid degeneration was very high for tendons with chronic tears in the myotendinous junction, and cellularity was high for chronic tears at the mid-substance, it can be concluded that degeneration and the presence of a tear are linked factors. Increased cellularity can be a result of inflammation, and lipoid degeneration could be due to muscle atrophy, both associated with tendon injury. Both degeneration parameters can serve as a potential reason for the high suture pull out rates in rotator cuff repairs. This study aims to address the high failure rates of surgical repair for a degenerative rotator cuff tear. This study is significant because high amounts of degeneration can result in reduced material properties, which can lead to a chronic tear and further tendon degeneration. This is clinically significant because surgeons should avoid areas of poor tissue quality when performing a rotator cuff repair. Although the results did not support the hypotheses, the results showed that increases in localized tendon degeneration are not limited to the medial edge of the tear. Future directions for this study include evaluating nuclei shape to further evaluate differences in localized degeneration.

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**REFERENCES**