Quantitative Ultrasound Elastography Can Quantify Mechanical Tendon Healing In A Rabbit Achilles Tendon Transection Model.

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Introduction: Quantitative evaluation of tendon healing after Achilles tendon rupture is clinically important to determine appropriate timing of return to sports and to prevent re-rupture. It is also useful to assess the effectiveness of new treatments such as platelet-rich plasma injection. However, there are no clinically available, non-invasive methods to quantify tendon healing. Elastography is an ultrasound technique to measure tissue elasticity. Several studies have shown that elasticity of the Achilles tendon measured with elastography changes over time after Achilles tendon rupture, and that elastography may be used to assess tendon healing. However, the qualitative evaluation was used in the previous reports. Additionally, no studies have examined correlation between the mechanical property of the Achilles tendon and elastography measurement. Recently quantitative elastography, strain ratio (SR) measurement, has been developed, and quantification of Achilles tendon elasticity using SR is shown to be a reproducible technique. The purposes of this study were 1) to measure time-dependent change in the SRs of the healing site in a rabbit Achilles tendon transection model, 2) to assess correlation between the SRs and mechanical property of the healing site.

Methods: Twelve-week-old skeletally mature female New Zealand white rabbits (n = 30) were experimented. Under standard aseptic conditions and anesthesia, a longitudinal incision was made over the posterior aspect of each right leg and the Achilles tendon was exposed. The tendon was transected at 2 cm proximal to the calcaneal insertion. Then the wound was closed in a layered fashion. After surgery, rabbits were allowed to move freely within their cages with no splinting. Six rabbits were sacrificed at 2, 4, 8 and 12 weeks after surgery for the ultrasound and mechanical evaluations. Additionally, non-operated twelve-week-old rabbits were sacrificed as a normal control.

For ultrasound evaluation, the previous surgical wound was opened under standard anesthesia. The surrounding soft tissue, including the plantaris tendon, was removed, and the Achilles tendon was exposed. Each Achilles tendon was examined in a standard prone position with the feet hanging over the edge of the examination bed. The longitudinal image with the center of the probe at 1.5cm proximal to the calcaneal insertion was evaluated with an ultrasound system (Hi Vision Preirus, Hitachi Aloka Medical, Japan) and the linear probe (6~14MHz). For quantitative elastography, an acoustic coupler with a gel pad of known Young’s module (22.6±2.2kPa) was attached on the probe. Repeated vertical pressure of approximately 2Hz was applied to the tendon manually using the probe. Color mapping of the tissue strain was superimposed on the B-mode image, ranging from red (soft), green/yellow (medium) to blue (hard). Then, regions of interest were defined for the Achilles tendon and the coupler. Strain value of the tendon (B) was divided by that of the coupler (A), and the strain ratio (SR) of the
tendon was calculated (B/A). Additionally, for the axial B-mode image, the cross-sectional area of the
tendon healing site was measured using the axial B-mode image. After the ultrasound evaluation, each
Achilles tendon was harvested with the calcaneus attached, and used for mechanical testing. All
measurements were performed three times by a single experienced orthopaedic surgeon, and the mean
of the three measurements were used.
For mechanical testing, the specimen was clamped using specially designed apparatus. The grips were
set at a distance of 40mm. Prior to loading, a 5N tensile preload for 5 minutes was applied to align the
specimen. The whole construct was loaded at 10 mm/minute velocity traction speed. Maximum load,
maximum stress, elastic modulus and linear stiffness. Elastic modulus was calculated between the points
corresponding to 20% and 80% of the maximum stress. Linear stiffness represented the slope of the
load-displacement curve between the points corresponding to 20% and 80% of the maximum load.
To assess intraobserver repeatability of the SR measurement, intraclass correlation coefficient (1, 3) was
determined using R2.8.1. Time-dependent changes in the SR values and mechanical parameters were
assessed using the one-way analysis of variance. Correlations between the SR and maximum load,
maximum stress, linear stiffness and elastic modulus were assessed using the Spearman's rank
correlation coefficient. Statistical significance was set at P < 0.05.
**Results:** Intraclass correlation coefficient (1, 3) of the SR measurement was 0.74, indicating substantial
intraobserver repeatability. The average SR of the healing site was 1.31±0.49 (average±standard
deviation) at two weeks after operation. The average SRs sequentially decreased, and Achilles tendons
were sequentially stiffer (P < 0.001). The average SR at twelve weeks was 0.45±0.17 and almost same
the SR of normal tendons (0.43±0.10) (Fig.1). The average maximum stress and elastic modulus at two
weeks were 2.8±1.5 and 17.3±6.5, respectively (Fig2a, b). The values significantly increased over time (P
= 0.019 and P = 0.034), and were 24.9±9.7 and 300.0±99.3 at twelve weeks. There were significant
correlations between the SR and maximum stress (p= 0.68, P < 0.001, Fig3a), and the SR and elastic
modulus (p= 0.74, P < 0.001, Fig3b). Correlations between the SR and maximum load (p= 0.68, P <
0.001), linear stiffness (p= 0.667, P < 0.001) were also significant.
**Discussion:** Quantitative elastography using the acoustic coupler (SR measurement) is a new ultrasound
technique to quantify tissue elasticity. We showed the SRs of the healing site decreased sequentially
after Achilles tendon rupture using a rabbit Achilles tendon transection model. De Zordo et al. measured
the elasticity of the human Achilles tendon using conventional qualitative elastography [1]. They
reported the Achilles tendon appeared a stiffer structured pattern in healthy volunteers. The
quantitative measurement in this study can be useful method to evaluate the healing after Achilles
tendon rupture.
We also showed significant correlations between the SR values and mechanical property of the healing
tendon. While Klauser et al. showed correlation between qualitative elastography and histological
tendon degeneration of the human Achilles tendon, this is the first study to assess correlation between
the elastography measurement and mechanical property of the Achilles tendon [2]. Although SR
represented elasticity against the transverse compression force, the value was significantly correlated
with the longitudinal mechanical property which is clinically important. Quantitative elastography may
be a useful method to quantify mechanical property of the tendon of after Achilles tendon rupture.
This study had several limitations. First, correlation between the SR and histological assessment, which is the other gold standard to evaluate tendon healing, was not investigated. Second, tendon transection model is different from clinical Achilles tendon rupture.

**Significance:** Quantitative elastography using the acoustic coupler can be a useful method to quantify tendon healing after Achilles tendon rupture.
Fig. 3

(a) Maximum stress (MPa) vs. Strain ratio
- $\rho = -0.68$
- $P < 0.001$
- 2w
- 4w
- 8w
- 12w
- Normal

(b) Elastic modulus (MPa) vs. Strain ratio
- $\rho = -0.74$
- $P < 0.001$
- 2w
- 4w
- 8w
- 12w
- Normal