Difference in the fibular attachment structure between the superior and inferior fascicles of the anterior talofibular ligament using ultrasonography and histological examinations

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Research Article

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Abstract

Purpose

The anterior talobular ligament (ATFL) is divided into superior (SB) and inferior bands (IB). Although the differences in length and width are known, the structure of the fibular attachment had not been elucidated. The present study aimed to clarify the differences in the fibular attachment structure between ATFL's SB and IB using cross-sectional images along the ligament.

Methods

An anatomical study using 15 formalin-fixed ankles was performed. The lateral ankle ligament complex was collected after a longitudinal image of SB/IB was visualized by ultrasonography. The specimens were decalcified and sectioned longitudinally at the center of SB/IB using a microtome. Histological evaluation of the enthesis structure at the fibular attachment of SB/IB was performed using hematoxylin–eosin and Masson's trichrome stains.

Results

A fibrillar pattern could not be observed in the longitudinal image at the IB level by ultrasonography. The lengths of ATFL's SB and IB were 20.6 ± 1.6 and 15.3 ± 1.3 mm, respectively, with thicknesses of 1.8 ± 0.4 and 1.0 ± 0.4 mm, respectively. The ATFL's IB was significantly shorter and thinner than the ATFL's SB. The fibular attachment of ATFL's SB had distinct enthesis structure, whereas in the attachment structure of the ATFL's IB, there were several variations including a type with a narrower enthesis structure than the ATFL's SB and a type that merged with or wrapped around the calcaneofibular ligament.

Conclusion

The fibular attachment structure between ATFL's SB and IB differs. Our results could be useful information when performing ultrasonography and MRI diagnosis.

Introduction

Ankle sprains are among the most common sports injuries that tend to damage the lateral ankle ligament complex, which is composed of the anterior talofibular ligament (ATFL), calcaneal fibular ligament (CFL) and posterior talofibular ligament. Among them, the ATFL is the part most vulnerable to ankle inversion sprains [18].

Although the number of ATFL fascicles (single, double, and triple/multiple) vary, the ATFL is often divided into two bundles, i.e. superior (SB) and inferior (IB) bands/fascicles [6, 9, 10, 14, 16, 17]. In case of a
double bundle, the branch of the lateral malleolus artery penetrated between the two fascicles [6, 14, 26], which allows clinicians to easily distinguish the two fascicles from each other. Both fascicles of ATFL were considered as the intracapsular ligament; recently, the SB is referred to as the intraarticular ligament, whereas the IB is the extraarticular ligament, and the IB was referred to as the lateral fibulotalocalcaneal ligament (LFTCL) complex with CFL [26]. There are variations in the attachment structure of the ATFL and CFL, ATFL has several types that connect to the CFL at the deep or superficial layer [17]. Our previous report demonstrated two types of IB structure: one was clearly the extraarticular type and the other was the integrated-capsular type, which integrated with the lateral-inferior capsule of the ankle joint [12].

The morphology of SB and IB can be evaluated by axial section using magnetic resonance imaging (MRI) [20, 23, 24], but it was not practical to perform MRI examinations on all patients with ankle sprains due to time-consuming and high medical cost. Recently, ultrasonography has been commonly used to evaluate the location and severity of ATFL injury [2, 11]. In cases of ATFL injury, the presence/absence of ligamentous injury can be easily determined if a fibrillar pattern longitudinal to the ATFL could be visualized by ultrasonography. Contrarily, evaluating the IB is difficult because visualizing the fibrillar pattern on the longitudinal image obtained by ultrasonography is difficult.

The footprint of the fibula attachment of SB was reportedly located in front of the obscure tubercle (OBT) [6, 19]. Although the fibular attachment of the IB is located below the OBT and is quite complex as it intersects with the CFL, the manner of how it attaches to the fibular has not been clarified. Moreover, the anatomical difference in the enthesis structure on the fibula between the two fascicles is still unclear.

The present study aimed to clarify the structure of the fibular side attachment of the ATFL’s SB and IB, using ultrasonography and histology of embalmed cadaver.

**Materials And Methods**

**Cadavers**

The present study used nine formalin-fixed Japanese cadavers [1 female and 8 male cadavers, mean age of 78.3 ± 9.2 (range 63–96) years] donated to Shinshu University School of Medicine’s Department of Anatomy. One ankle was eliminated due to the presence of a lateral ankle ligament injury, two ankles had been used in another anatomical study. Thus, in this study, 15 ankles were used (7 right and 8 left ankles).

**Ultrasonography finding**

Longitudinal images at the ATFL’s SB and IB of four intact ankles were taken using ultrasonography (SONIMAGE MX1, KONIKA MINOLUTA, in Japan) with a 3–11 MHz linear probe. The ankle joint was placed in a mild plantar flexion (approximately 20°) position. After palpating the OBT at the lower end of the fibula, the SB was palpated and the fibrillar pattern was visualized by ultrasonography. Imaging of the IB was performed by translating the probe from the site of visualization of the SB toward the lower end of
the fibula. To confirm whether the longitudinal positions at the center of the ATFL's SB and IB were accurately depicted, the skin and connective tissue were peeled off and the probe position was confirmed after ultrasonographic imaging.

**Study protocol**

The lower leg was amputated 10 cm above the lateral malleolus and the ATFL was carefully dissected, as described elsewhere [13]. The ATFL of the 14 ankles showed a double bundle, which was divided into SB and IB (Fig. 1). The length and thickness of ATFL's SB and IB were measured with a digital caliper. One ankle was used only for cross-sectional histological study without skin and soft tissue dissection. The length of the SB and IB were measured as the distances to the attachment of the fibula and talus from outside the capsule, respectively. Using an electric bandsaw (PROXXON No.28170, Osaka, Japan), the ankles were cut along the ATFL at the upper margin of the SB and the lower margin of the IB including the bone. Furthermore, at the site where the branch of the anterior lateral malleolus artery is located, which is the boundary between the SB and IB, the ATFL specimens were divided into the SB and IB band. The thickness of SB and IB was measured at the center of each fiber. Both specimens were decalcified in a decalcifying solution (K-CX AT, FALMA Co., Tokyo, Japan) for a week, as described in a previous study [13]. After decalcification, each specimen was obtained at the section along the center of each fascicle with a cryomicrotome, and the bone attachment of ATFL was observed under a stereomicroscope (NIKON SMZ1000, Tokyo, Japan) and photographed with a digital camera (EOS Kiss M2, CANON, Tokyo, Japan).

**Histological observation**

The specimens were fixed in 4% buffered formaldehyde and embedded in paraffin, and longitudinal sections along the ligament sliced at the center of each band to a thickness of 10 µm with a microtome were obtained. The specimens were stained with hematoxylin–eosin and Masson's trichrome stains. The structure of the ATFL fiber and attachment with the fibula and talus were visualized using a microscope (Keyence, BX-9000, Japan). In particular, we carefully observed the enthesis structure of the fibular attachment of the ATFL's SB and IB.

**Statistical analysis**

The Shapiro-Wilk test was used to confirm whether the data were normally distributed. The student's t-test was used to compare the fiber lengths and thickness between the ATFL's SB and IB. All data were analyzed using the Statistical Package for the Social Sciences (SPSS ver. 21 for windows; SPSS, IBM). Differences were considered significant at a P value of < .05.

**Results**

An ultrasound scan is shown in Fig. 2. The longitudinal image at the SB level of ATFL showed a clear fibrillar pattern. Contrarily, at the IB level, the fibrillar pattern around the talus could be visualized, but that on the fibular side could not. The length and thickness of the ATFL's SB and IB is shown in Table 1. The lengths of SB and IB were 20.6 ± 1.6 and 15.3 ± 1.3 mm with thicknesses of 1.8 ± 0.4 and 1.0 ± 0.4 mm,
respectively. The IB of the ATFL was significantly shorter and thinner than the SB. Figure 3 shows longitudinal cross-sectional images of the SB and IB taken with a stereomicroscope. The fibular insertion of SB was clearly visible, but the IB was difficult to distinguish due to the location of the CFL. A histological image of the longitudinal section at the center of the SB and IB is shown in Figs. 4 and 5. The attachment of the SB had a deep/wide region of bone, and a clear enthesis structure was observed, whereas the IB insertion to the fibula was shallow/narrow (Figs. 4 and 5). In some cases, the enthesis structure of the IB at the fibular side was not clear, and the IB converged with CFL (Fig. 4) or was covered the CFL (Fig. 5).

Table 1
Length and thickness of the superior (SB) and inferior (IB) bands of the anterior talofibular ligament (ATFL)

<table>
<thead>
<tr>
<th>ATFL SB</th>
<th>ATFL IB</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>Length</td>
<td>20.6 ± 1.6</td>
<td>15.3 ± 1.3</td>
</tr>
<tr>
<td>Thickness</td>
<td>1.8 ± 0.4</td>
<td>1.0 ± 0.4</td>
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Discussion

The most important finding of this study is that the difference in ligamentous structure between the SB and IB of the ATFL was clarified by examining longitudinal cross-sections from cadavers. The SB was longer and thicker than the IB and had a distinct fibrous structure. At the attachment on the fibular side, the SB had a clear enthesis structure, consisting of the ligament, non-calcified fibrocartilage, calcified fibrocartilage, and bone. Contrarily, the IB had a narrow area of the enthesis structure and covered or converged with CFL running a longitudinal direction.

Most of the previous studies on the difference in the morphology between the SB and IB of the ATFL have reported on the length and width of each fascicle [9, 17, 21, 28]. Additionally, some studies also determined the thickness of each fascicle [16, 27]. The present study also confirmed that the IB is a shorter and thinner fascicle as compared to the SB. By showing a longitudinal image of the central portion of each fascicle, it is easy to imagine the morphological structure of both fascicles. The SB attaches anteriorly to the OBT at the lower end of the fibula and runs straight to the talus. Contrarily, the IB attaches to the slope at the lower end of the fibula below the OBT and the fibular side is positioned slightly deeper, resulting in a shorter length.

ATFL was known to have fibers that merge with CFL extraarticularly [7, 8, 13, 14, 22, 28], and various types of confluence in the superficial and deep layers had been reported [17]. The fiber that connects the ATFL and CFL transmits the tension of both ligaments to each other [7]. Recently, the LFTCL complex was introduced and described as a complex structure in which the IB of ATFL and CFL merge
extraarticularly. In our previous report, ATFL and CFL confluence fibers were located in the superficial connective tissue, and when this connective tissue layer was removed, the footprint position of the ATFL and CFL became independent [14]. Additionally, there were two types of confluence between the ATFL’s IB and CFL, which are as follows: the LFTCL type where the IB and CFL merge extraarticularly, and the type where the deep layer of the IB was integrated into the inferior-lateral capsule and attached to the fibula [12].

Given that there were variations on the fibular side of IB, the morphology of the IB attachment had not been clarified. In this study, from the histological findings of the longitudinal section along the ligament, the fibular side of the SB had a distinct enthesis structure. Contrarily, there were variations in the fibular attachment of the IB, a type that covered or merged with the CFL, LFTCL complex, and a type that attached to the fibula and had an enthesis structure, but the area was smaller/shallower than that of the SB. The SB might be more important than the IB in terms of ankle control function based on the result of the analysis of the enthesis structure of the fibula. The difference in the attachment structure between th SB and IB could be explained by the fact that the lateral malleolus avulsion fracture occurred at the attachment of SB.

The SB and IB could be clearly visualized using a transverse section of a MRI scan [5, 20, 23]. MRI also showed that the IB had a shorter and thinner structure than the SB. However, because the IB has a narrow average width of 3.9 mm [26], it was difficult to visualize using a normal 3.0-mm slice thickness. MRI with 0.8-mm slice thickness was reported to be useful for the evaluation of the two fascicles, and the sensitivity and specificity of detecting the LFTCL injury with MRI were high [20]. In a previous study that evaluated ankle ligament injuries using 3D isotropic proton density-weighted fast spin-echo MRI, the most common type of injuries was a tear in both SB and IB, but, in rare cases, either the SB or IB was injured. Therefore, it was necessary to evaluate each fascicle separately [5].

Ultrasonography has become the gold standard for the diagnosis of ankle sprains due to its non-invasive and real-time assessment of ATFL [1–3, 25]. The methods for classifying the degree and state of ATFL damage into five or six types by ultrasonography had been reported, but they are evaluations of SB and the presence or absence of damage to the IB was not evaluated [4, 15]. The reason why it was difficult to visualize the IB in the longitudinal section may be due to fact that the IB is narrow and crosses the CFL on the fibular side. Additionally, because the IB adheres to the slope of the inferior fibula below the OBT, it was difficult to apply the probe parallel to the IB. When evaluating the LFTCL complex including the IB by ultrasonography, it was necessary to evaluate the signs of inflammation not only on the longitudinal images but also on the short-axis images. In this study, it was possible to clarify the difference in the attachment structure between the SB and IB of the ATFL, which could not be evaluated by ultrasonography, and we consider that our data would be useful during imaging examinations.

This study had several limitations. First, we only used 15 ankles of formalin-fixed elderly cadavers, even though ankle ligament injury is a sports injury in young people. However, in this study, we focused only on the morphological structure of ATFL and did not consider age and sex. Second, the morphological
structure of the SB and IB was visualized using the longitudinal section of only the central part of each fascicle. Although the IB had many structural variations and structural differences between the upper and lower margins of the IB might be present, this study clarified the structural characteristics of the central part of each fascicle. Third, since this study was an anatomical and histological experiment, it clarified only the structural characteristics of the ATFL’s fascicles, not the functions of the ATFL’s SB and IB. However, the strength of each fascicle could be estimated from the difference in the enthesis structure of the attachment; thus, based on our findings, it was thought that IB had a lower joint control function than SB.

**Conclusion**

There were differences in the structure of the longitudinal section along the ligaments of the SB and IB of the ATFL. In the attachment of the ATFL on the fibular side, the SB had a clear enthesis structure, whereas the enthesis structure was not clear and the attachment area to the fibula was narrow in the IB. Some types of ATFL’s IB converged or covered the CFL. The image of the longitudinal-sectional structure of the ATFL’s SB and IB in this study can be used as a reference when performing ultrasonography. Because it is difficult to depict the fibrillar pattern in the long-axis image of the ATFL’s IB by ultrasonography, it is necessary to evaluate it together with the short-axis image.

**Declarations**

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**Authors' contributions**

A Kakegawa: Study design, Data collection, Data analysis, Manuscript writing

N Fukushima: Histological experiment, Data analysis, Manuscript writing

N Sumitomo, A Nagira and Y Ichinose: Histological experiment, Critical revisions to the manuscript

All authors read and approved the final manuscript prior to submission.

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**Availability of data and materials**

Please contact corresponding author for data and materials.
Declarations

Competing interests

All authors declare that have no conflict of interest regarding this study.

Ethical Approval

This study protocol was approved by the Institutional Review Board of Shinshu University, Matsumoto, Nagano Prefecture, Japan (Approval No.4340) and Teikyo Hisei University, Toshima-ku, Tokyo, Japan (Approval No. R01-022)

Consent to participate

Informed consent of this anatomical study was obtained from the families of the donated cadavers.

References


Figures
Figure 1

Lateral view of the right ankle. The skin, extensor retinaculum, superior fibula retinaculum, fibula tendon sheath, and connective tissue on the lateral side are removed. The anterior talofibular ligament (ATFL) is divided into two fascicles: superior (SB) and inferior (IB) bands. LM: lateral malleolus, SB: superior buan of ATFL, IB: inferior band of ATFL, PB: peroneal brevis, PL: peroneal longus
Figure 2

The longitudinal ultrasonography image of the anterior talofibular ligament (ATFL) of the right ankle of a 73-year-old cadaver. a: Longitudinal image of the superior band (SB) of the ATFL. The ATFL fibrillar pattern can be observed clearly in the insertion from the fibula (arrowhead) to talus (arrow). B: Longitudinal image of the inferior band (IB) of the ATFL. The fibrillar pattern can be visualized on the talus side (double arrowhead), but cannot be clearly observed around the fibula. F: fibula, T: talus
Figure 3

The stereographic photographs of the longitudinal section of the anterior talofibular ligament (ATFL) at the superior (SB, a, c) and inferior (IB, b, d) band level. (a, b) The right ankle of a 73-year-old man. (c, d) The left ankle of a 63-year-old man with skin and soft tissue. Because the IB crosses the calcaneofibular ligament (CFL) (*) at the lower end of the fibula, the fibula attachment of the IB is less distinct than that of the SB. F: fibula, T: talus
Figure 4

The left ankle of a 75-year-old man. (a, b) Stereomicroscopic photographs of the longitudinal section of the anterior talofibular ligament (ATFL) at the superior (SB, a) and inferior (IB, b) band level. (c-f) Histological photographs of a longitudinal slice at the SB (c, e) and IB (d, f) level with hematoxylin and eosin stain (c, d) and Masson-trichrome stain (e, f). The fibula and talus attachments of the SB show a wide and firm enthesis structure (arrow, c, e), while the fibula-side attachments of the inferior fibers were
narrow/shallow and the enthesis structure was not clear (arrowhead, e, f). Dot circle: calcaneofibular ligament, F: fibula, T: talus, bar: 1 mm

Figure 5

The left ankle of 77-year-old man. (a, b) Stereomicroscopic photographs of longitudinal section of anterior talofibular ligament (ATFL) at superior (SB, a) and inferior (IB, b) bands level. (c-f) Histological photographs of a longitudinal slice at SB (c, e) and IB (d, f) with hematoxylin and eosin stain (c, d) and Masson-trichrome stain (e, f). The fibula and talus attachments of the SB show a wide and firm enthesis
structure (arrow, c, e), while the fibula-side attachments of the inferior fibers were narrow/shallow and the enthesis structure was not clear (e, f). The fibular side of IB (d, f) is attached to the fibula to cover or converged with calcaneofibular ligament (arrowhead). dot circle: calcaneofibular ligament, F: fibular, T: talus, bar: 1 mm