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What is This?
Anterolateral Ligament of the Fetal Knee

An Anatomic and Histological Study

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Investigation performed at the University of São Paulo, São Paulo, Brazil

Background: The anterolateral ligament (ALL) of the knee has recently been described in detail. Most studies of the ALL have been conducted in adults; therefore, little is known about the anatomy and histology of the ALL in younger patients, and nothing is known about the fetal presence of the ALL.

Purpose: To evaluate the ALL in human fetuses to determine its presence or absence and to describe its microscopic anatomy and histological features compared with the findings of studies conducted in adults.

Study Design: Descriptive laboratory study.

Methods: Twenty human fetal cadaveric specimens were used. The mean age of the fetuses was 28.64 ± 3.20 weeks. The ALL was dissected in the anterolateral region of the knee, and its anatomic parameters, including its origin, insertion, and path in relation to known adjacent anatomic landmarks, in addition to its length, width, and thickness over the path toward the tibia, were measured. After dissection, the ALL was removed en bloc with a portion of the lateral meniscus for histological analysis of 4-μm sections, hematoxylin and eosin staining, and immunohistochemical staining for type I collagen.

Results: The ALL was located in all dissected knees. Its origin was located at a mean distance of 1.87 mm from the origin of the lateral collateral ligament, with variations from the center of the lateral epicondyle to posterior and proximal to it, and it exhibited an anterior-inferior path toward the tibia, an insertion in the lateral meniscus approximately 2.08 mm anterior to the popliteal tendon, and another insertion in the tibia between the Gerdy tubercle and the fibular head at 2.46 mm below the articular cartilage. The histological sections of the ALL showed well-organized, dense collagenous tissue fibers with elongated fibroblasts (mean, 1631 fibroblasts/mm²) and a predominance of type I collagen.

Conclusion: The ALL is present during fetal development, with anatomic and histological features similar to those of the adult ALL.

Clinical Relevance: The findings of this study help to better understand the ALL’s anatomy and histology from the fetal period to adulthood. The study presents the existence of the ALL since fetal development, emphasizes the characterization of the ALL, and brings important information to future pediatric ALL lesion studies.

Keywords: knee ligaments; anatomy; anterolateral ligament; ACL; fetal anatomy; histology
the Gerdy tubercle, approximately 5 to 10 mm distal to the lateral plateau in adults. In addition to this tibial insertion point, several researchers have found an insertion of the ALL in the lateral meniscus, described in detail in a study by Helito et al.8

The ALL is biomechanically important in the control of rotational stability of the knee, functioning as a secondary restrictor to the anterolateral rotation of the tibia in relation to the femur, especially in cases of associated injuries to the anterior cruciate ligament (ACL).17-19 There is also controversy regarding the level of knee flexion at which the ALL has more tension and therefore produces greater action.2,3,12,26

Most studies of the ALL have been performed on adults and, because of the availability of anatomic specimens, have most involved elderly patients; thus, little is known about the anatomy and histology of the ALL in younger patients, and nothing is known about fetal ALL development. A single study conducted by Shea et al21 attempted to describe the ALL in patients with an immature skeleton, but the authors only managed to isolate this structure in 1 of the 8 cases studied. These researchers suggested that the ALL may not be fully developed in the immature skeleton.

The anatomy of the ACL in developing fetuses was described in detail by Ferretti et al5 using 40 anatomic specimens with a mean age of 20 weeks. That study was important because it described the development of the ACL, the presence of its 2 anatomic bands, and the similarities and differences to the adult ACL. Therefore, the main objective of the present study was to evaluate the ALL in human fetuses to determine its presence or absence and describe its macroscopic anatomy and histological features relative to the findings obtained in studies of adults. The secondary objective was to assess its behavior during knee flexion and extension.

METHODS

In this study, 20 human fetal cadaveric specimens were used. The fetuses were obtained from preserved cadaveric specimens of the Department of Anatomy of our institutions. The study was approved by the Committee of Research and Education of the University of São Paulo. The age of the fetuses varied from 25.5 to 37.3 weeks, with a mean of 28.64 ± 3.20 weeks, and the mean fetal length was 39.69 ± 4.33 cm. Specimens with malformations were excluded from the study. The fetuses were preserved in 10% formalin. Ten right knees and 10 left knees were used, of which 10 were from male specimens and 10 were from female specimens.

Basic dissection materials were used to dissect the fetuses. First, the skin of the thigh and leg was circumferentially incised approximately 4 cm proximal and 4 cm distal to the knee joint line. A transverse midline incision connecting the 2 initial incisions was then performed in the anterior region of the knee. A subfascial dissection plane was followed, detaching all skin circumferentially. Next, the fascia lata and femoral biceps tendon were dissected and incised approximately 3 cm proximal to the joint line. These structures were carefully dissected up to their distal insertions to maintain their insertion in the Gerdy tubercle and the fibular head, which served as anatomic landmarks for subsequent measurements. Then, the LCL was carefully dissected. When all bone parameters were easily visible, the ALL was carefully dissected. The ALL was observed more easily during knee flexion and internal rotation of the tibia, a position in which it had apparently more tension. All dissections followed the same protocol. After isolating the ALL, measurements were performed using a digital caliper with 0.01-mm precision and a 10× magnifying glass for precise viewing and measurement of the structures of interest. The measurements (in mm) were performed separately by 2 authors (J.A.P.T. and J.A.A.).

The length of the ALL was measured with the knee in full extension and at 30°, 60°, and 90° of flexion, with always with neutral rotation of the knee. The width and thickness of the ALL were also measured at its origin, at the level of the joint line, and at the tibial insertion. The femoral origin of the ALL was measured in relation to the lateral epicondyle and LCL. The tibial insertion of the ALL was measured in relation to the Gerdy tubercle, the fibular head, and the lateral plateau. The meniscal insertion of the ALL was measured in relation to the popliteus muscle tendon at the height of the joint line. The size and macroscopic shape of the ALL insertion sites in the femur and tibia were also evaluated.

After dissection and measurement of anatomic parameters, the ALL was removed en bloc along with a portion of the lateral meniscus because of difficulty in totally isolating the structure resulting from the small size of the fetal structures. The ligaments were removed and used for histological analysis.

Histological analysis was performed by placing the dissected material in 10% formalin for tissue fixation. After standard processing, 4-μm histological sections were cut and stained with hematoxylin and eosin, and immunohistochemical staining was performed for type I collagen (1:1000 dilution; Clone ab34710; Abcam). The fibroblast count was determined in the fetal structure and then compared with the count of previously dissected adult anatomic specimens.

Statistical Analysis

Statistical analysis was performed to evaluate the interobserver agreement between the anatomic measurements performed by the 2 evaluators and to evaluate the alteration in length between different degrees of knee flexion. The intraclass correlation coefficient (ICC) was used for the interobserver agreement, and the Student t test was performed to analyze the change in length between different degrees of flexion.

RESULTS

Results are reported as means ± SDs. The ALL was observed clearly in all of the 20 dissected knees. At the time of dissection, with internal rotation of the tibia and knee flexion, the ALL was observed below the iliobibial tract, immediately anterior to the LCL.

The femoral point of origin of the ALL exhibited a close relationship to the origin of the LCL, located at a mean of 1.87 ± 0.81 mm from the central point of origin of the LCL.
(Figure 1). Some variation was found in the point of origin of the ALL. Because the origin of the LCL is located at the posterior and proximal portion of the lateral epicondyle, according to the findings of LaPrade et al., the ALL was located near the center of the lateral epicondyle (LFE) and an anterior-inferior path toward the tibia, with the tibial insertion between the Gerdy tubercle (GT) and the fibular head (FH). BT, biceps tendon; LFC, lateral femoral condyle.

Following its origin in the femur, the ALL followed an anterior-inferior path toward the tibia. At a mean point of 57.7% ± 15.4% of its path, the ALL exhibited a bifurcation toward the lateral meniscus, with an insertion in the transition between its body and the anterior horn. The meniscal insertion occurred at a mean of 2.08 ± 0.80 mm anterior to the popliteal tendon at the joint level, when the latter was intra-articular (Figure 3). The main portion of the ALL continued along a path toward the tibia, with mean insertions of 6.29 ± 1.62 mm posterior to the posterior edge of the Gerdy tubercle, 6.61 ± 2.13 mm anterior to the anterior edge of the fibular head, and approximately 2.46 ± 0.78 mm below the lateral articular cartilage.

**Figure 1.** Anatomic image of a 26.6-week-old fetal left knee showing the anterolateral ligament (ALL) (asterisk), anterior to the lateral collateral ligament (LCL), with an origin near the center of the lateral epicondyle (LFE) and an anterior-inferior path toward the tibia, with the tibial insertion between the Gerdy tubercle (GT) and the fibular head (FH). BT, biceps tendon; LFC, lateral femoral condyle.

**Figure 2.** Anatomic image of a 28.4-week-old fetal left knee showing fibers of the origin of the anterolateral ligament (ALL) (asterisk), near the origin of the lateral collateral ligament (LCL), passing over its origin toward the tibia. BT, biceps tendon; FH, fibular head; GT, Gerdy tubercle; LFC, lateral femoral condyle; LFE, lateral epicondyle.

**Figure 3.** Anatomic image (internal view) of the lateral portion of a 25.5-week-old fetal right knee showing the relationship of the anterolateral ligament (ALL) to the popliteal tendon (PT), forming a triangle with a base at the lateral meniscus and a vertex at the lateral epicondyle. LFC, lateral femoral condyle; M, lateral meniscus.
The mean lengths of the ALL were 7.19 ± 1.86, 7.60 ± 1.79, 8.52 ± 1.55, and 9.11 ± 1.60 mm with the knee in full extension and at 30°, 60°, and 90° of flexion, respectively (Table 1). Therefore, an increase in the ALL length was observed with knee flexion. The mean increase from extension to 30°, 30° to 60°, and 60° to 90° of flexion was 0.41, 0.92, and 0.59 mm, respectively (P < .05 for all) (Table 2).

The mean ALL widths were 2.14 ± 0.57, 2.12 ± 0.47, and 2.67 ± 0.68 mm at the origin, joint line, and tibial insertion, respectively. The mean thicknesses of the ALL were 0.45 ± 0.16, 0.39 ± 0.16, and 0.56 ± 0.18 mm at the origin, joint line, and tibial insertion, respectively. After en bloc resection of the ALL, a mean circular femoral footprint of 3.85 ± 1.91 mm² and a mean band-shaped tibial footprint of 1.83 ± 1.42 mm² were observed (Figure 4). The ICCs for the variables studied ranged from 0.87 to 0.97.

The histological sections of the ALL showed dense, well-organized collagenous fibers with elongated fibroblasts (mean, 1631 fibroblasts/mm²). The fetal ALL had an increased cell concentration compared with the adult ALL (mean, 121 fibroblasts/mm²) and was formed predominantly of type I collagen (Figure 5). Differences between male and female specimens are shown in the Appendix (available online at http://ajsm.sagepub.com/supplemental).

**DISCUSSION**

The main finding of this study was that the ALL is a structure that is present during fetal development, with anatomic and histological features similar to the ALL of adults. The ALL was found in all of the 20 cadaveric specimens used in this study, similar to recent studies that clearly identified the ALL in most of the specimens studied. In a recent systematic review, the ALL was characterized in 96% of the cases studied in 16 articles. Nevertheless, studies such as that conducted by Dombrowski et al found well-defined lateral capsular thickening in only 40% of the cases.

The position and path of the ALL in relation to the neighboring structures observed in the fetuses were consistent with the recent literature regarding this structure. The most controversial anatomic parameter was the correct femoral point of origin of the ALL. While Helito et al and Claes et al located the ALL anterior and distal to the LCL, near the center of the lateral epicondyle, Dodds et al, Kennedy et al, and Lutz et al observed the ALL posterior and proximal to the lateral epicondyle. Caterine

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<th>Flexion, deg</th>
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<tr>
<td>0°</td>
<td>7.19 ± 1.86</td>
<td>6.90</td>
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<tr>
<td>30°</td>
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<tr>
<td>90°</td>
<td>9.11 ± 1.60</td>
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<table>
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<th>P Value</th>
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![Figure 4](image4.png) Schematic drawing showing the anterolateral ligament (ALL) femoral footprint and the band-shaped ALL tibial footprint.

![Figure 5](image5.png) Histological images of (A) the anterolateral ligament (ALL) and its relationship to the lateral meniscus (M) in a 37.3-week-old fetus, showing (B) the presence of intra-substantial blood vessels (scale bar = 100 μm), (C) well-organized and dense connective tissue with high fibroblast cellularity (scale bar = 50 μm), and (D) an abundant presence of type I collagen (scale bar = 50 μm). The asterisk in (A) indicates the distal portion of the fetal ALL.
et al\textsuperscript{1} found both variations. Vincent et al\textsuperscript{25} were the only investigators to determine a closer relationship of the ALL to the popliteal tendon. In a different anatomic study, Helito et al\textsuperscript{11} were unable to differentiate the origin of the ALL and LCL in 2 cases and found posterior and proximal variation in a minority of the dissected knees (6.2\%). In our study, the ALL was found anterior to the LCL in most knees, near the center of the lateral epicondyle or slightly posterior and proximal to it and not anterior to it, although we also observed anatomic variations. We propose that the variations among studies may be related to the difficulty of dissecting the lateral region of the knee, which includes the possible removal of a small amount of tissue that may be proximal to the origin of the LCL, and the possibility that the origin of the ALL has extensions that may have greater or lesser anatomic and biomechanical significance. Kawaguchi et al\textsuperscript{13} and Smigielski et al\textsuperscript{22} showed that even greater or lesser anatomic and biomechanical significance.

The relative length measurements between flexion angles found in this study are consistent with the findings of biomechanical studies conducted by Helito et al\textsuperscript{12} and Zens et al\textsuperscript{26} showing an increase in the ALL length during knee flexion. Dodds et al\textsuperscript{15} observed the opposite results, with increased length upon knee extension. These differences may be related to the point of origin of the ALL in the femur. However, Kennedy et al\textsuperscript{14} found a femoral point similar to Dodds et al,\textsuperscript{15} but with behavior similar to that of studies showing greater length during knee flexion, indicating a lack of consensus in the literature regarding ALL behavior. Theoretically, a proximal and posterior point of origin would produce ALL behavior opposite to that of a distal and anterior origin. We believe that a greater length during flexion suggests ALL tensioning during flexion. Even though some structures were removed to isolate the ALL, we do not believe it to have significantly altered the physiological joint mechanics. A biomechanical study by Parsons et al\textsuperscript{19} showed that the action of the ALL is more significant in flexion angles greater than 35°, although a pivot shift, attributed to ALL failure, occurs at angles closer to extension. Understanding the behavior of the ALL throughout the range of motion is important for defining the ideal position for tensioning the structure during reconstruction techniques. Currently, there is no consensus in the literature regarding how possible fixation should be performed.\textsuperscript{6,23} The main variations of the structure include the main point of origin of the ligament in the femur and the degree of knee flexion during ligament tensioning and fixation.

The fetal ALL was 6 times shorter, 4 times narrower, and approximately 4 times thinner than the adult ALL dimensions reported in the literature. This variation was slightly lower than the variation found in a study of the fetal ACL; however, Ferretti et al\textsuperscript{5} used fetuses with mean age of 20 weeks, whereas the fetuses used in our study had a mean age of 28.64 weeks, which may explain this difference. The differences in measurements found between male and female specimens can also be explained by the differences in fetal age and length. Female specimens were a mean of 1.20 weeks older and 2.18 cm taller than male specimens; that is probably why most of the measurements presented higher values in female specimens.

Regarding histological analysis, the studied structure consisted of dense, well-organized connective tissue, similar to the ligament structure found in adults, but with increased cellularity. The cell count (per mm\textsuperscript{2}) in this study was lower than that found by Ferretti et al\textsuperscript{25} in their study of the fetal ACL (5600 × 1631 per mm\textsuperscript{2}), which may also be explained by the difference in the mean age of the fetuses between the studies. An alternative hypothesis is that the ALL may contain fewer cells than the ACL. Markers of type I collagen, the main collagen in ligaments, were strongly positive. All these findings (collagen type, organization pattern, cellularity) confirm the presence of a true ligament structure in the region in addition to capsular tissue. ALL bifurcation was clearly observed in the slides analyzed. The lateral inferior genicular vessels observed can act as guides for locating the ALL in magnetic resonance imaging scans, as suggested by Helito et al\textsuperscript{11} in an imaging study of the normal ALL.

According to our findings, we do not agree with the conclusions of Shea et al\textsuperscript{21} who proposed that the ALL is an inconstant structure in the pediatric population and that it only develops after the anterolateral capsule of the knee is subjected to physiological loads. According to the dissections performed, the ALL is already present in fetuses with a mean age of 28.64 weeks and exhibits development similar to other ligaments in the knee region, such as the ACL.

The limitations of this study include the use of cadaveric specimens preserved in formaldehyde instead of fresh cadaveric specimens, which could have possibly significantly altered the anatomic and histological properties of the specimens. The number of cadaveric specimens was also low, but we believe that 20 specimens was sufficient to validate our findings based on recent anatomic studies. The age range of the fetuses, from 25.5 to 37.3 weeks, may also be considered a limitation, but based on the difficulty of obtaining fetal tissue, we believe that it did not interfere with the main results and conclusions of our study. The fact that we removed some structures to isolate the ALL may have altered the physiological joint mechanics, which could have slightly altered the measurements found during flexion and extension, although we believe that tendency was not compromised.

**CONCLUSION**

The ALL is a structure that is present during fetal development, with anatomic and histological characteristics similar to those of the adult ALL. The ALL increased in length with knee flexion.
REFERENCES


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