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Synovium and Blood Capillaries at the Femoral Attachment of Anterior Cruciate Ligament are Significantly Abundant than those at the Tibial Attachment

Sinovial y Capilares Sanguíneos en la Inserción Femoral del Ligamento Cruzado Anterior son Significativamente más Abundantes que los de la Inserción Tibial

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SUMMARY: The anterior cruciate ligament (ACL) is a ligament that mainly controls the anterior and rotational mobility of the knee joint, and its surface is covered by a synovial membrane with large number of blood vessels. In general, nutritional supply to the ligament is from many capillaries in the adjacent synovium. However, statistical studies of the capillaries distributed to the ACL are insufficient. In this study, we examined cross-sectional histological images of the femoral attachment (femoral level), middle level of the tendon (middle level), and tibial attachment (tibial level) of the ACL and statistically analyzed blood capillary distribution among the three levels. The ACLs of 10 cadavers were divided into 5 equal sections, and 4mm-thick paraffin sections were made at the femoral level, middle level, and tibial level, and then hematoxylin-eosin (HE) staining were performed. The area of each transverse section was measured using Image-J 1.51n (U. S. National Institutes of Health, Bethesda, MD, USA). Fiber bundles of the ACL were relatively small and sparse in cross-sectional area at the femoral level and became larger and denser toward the tibial level. Many blood levels. The synovium at the attachment of ACL covered the surface of the fiber bundle and also penetrated deeply between the fiber bundles. In particular, the blood capillaries were densely distributed in the synovium at the femoral attachment rather than another two levels. Indeed, the number of capillaries were also most abundant in the femoral level. The cross-sectional ACL area at the femoral level is significantly small, however, the blood capillaries were most abundant. Therefore, when the ACL is injured, its reconstruction with preservation of the femoral ligamentous remnant may be clinically useful for remodeling of the grafted tendon.

KEY WORDS: Anterior cruciate ligament; Blood capillary; Synovium; ACL reconstruction; Remodeling.

INTRODUCTION

The anterior cruciate ligament (ACL) is covered by a layer of synovial tissue and runs from the lateral femoral intercondylar fossa to the anterior tibial intercondylar region, twisting posterolaterally and distally anteriorly. The ACL mainly controls the anterior and rotational mobility of the knee joint. When the knee is extended, the ACL has a mean length of 32 mm and a width of 7–12 mm and longer in males than in females (Duthon *et al*., 2006). The femoral attachment is round or oval and the size can vary from 11 to 24 mm across (Duthon *et al*., 2006). The tibial attachment is approximately 17mm in anteroposterior diameter and approximately 11mm in lateral diameter (Duthon *et al*., 2006). The cruciate ligaments get their major blood supply from the middle and inferior geniculate arteries. Those arteries give branches to the synovial tissue that covers the cruciate ligaments. From the synovial tissue, small blood

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vessels penetrate the ligament substance in a centripetal direction and anastomose with the longitudinally orientated intraligamentous vessels (Petersen & Tillmann, 1999). When the ACL is injured, the nonunion rate of the injured ligament is 40-100 % (Nayak *et al*., 2020). ACL insufficiency can cause meniscus and joint damage (Everhart *et al*., 2020; Lee *et al*., 2020), leading to irreversible osteoarthritis.

The reconstructive surgery using autologous tissue as the reconstructive ligament is often indicated. In Japan, approximately 13,000 ACL reconstructions are performed annually, In the United States, approximately 350,000 ACL reconstructions are performed annually, and it is estimated that approximately 1 million ACL reconstructions are performed annually worldwide (Sugimoto *et al*., 2016; Davies *et al*., 2017). Autologous graft tissues used include the hamstring, bony patellar tendon, and quadriceps tendon, and many good results have been reported (Koga *et al*., 2014; Amano *et al*., 2015; Terauchi *et al*., 2016). On the other hand, remodeling of grafts takes a long time, making early return to sports difficult (Terauchi *et al*., 2016).

It is believed that by leaving ligamentous remnant tissue at ACL attachments during its reconstruction, the blood vessels within the tissue promote remodeling and revascularization of the grafts (Berruto *et al*., 2014; Middleton *et al*., 2014). On the other hand, leaving the remnant tissue may increase the difficulty of the surgery, such as poor intraoperative vision, difficulty in identifying the anatomical markers and bone tunnel malposition at the time of bone tunnel creation. Although there have been many gross anatomical and histological observations of the blood vessels supplying the ACL and the adjacent tissues (Petersen & Tillmann, 1999; Lankes *et al*., 2000), the statistical evaluation of the vascular distribution throughout the ACL is still lacking. Therefore, in this study, we performed statistical analysis of the vascular components supplying the ACL at the femoral, middle and tibial levels and discuss the usefulness of preservation of the ligamentous remnant for ACL reconstruction.

MATERIAL AND METHOD

Subjects and Gross Anatomy. The study was designed in conformity with the Guidelines for Cadaver Dissection in Clinical Education and Research (as revised in 2016) and was approved by the Ethics Committee of Tokyo Medical University (Ethics Review Number: T2020-0050).14 ACL specimens were randomly selected from 10 cadavers [mean age, 82 years (range, 68-92 years); 7 male, 3 female] that were embalmed formalin, who had no history of surgery, including ligaments reconstruction or knee arthroplasty. In order to observe the running of the middle and inferior genicular arteries, which are the nutrient vessels to the ACL, dye resin (SKYPRENE® latex LA-502, polymo red FFB: KIWA CHEMICAL WAKAYAMA JAPAN) was injected from the popliteal artery, and gross anatomic observation was performed (Fig. 1).

Histological study. The sampled ACLs were decalcified by 0.5mol/l-EDTA for a month. They were divided into 5 equal parts (Fig. 2), and they were re-fixed in formalin (10 %) and embedded in paraffin. Sections of 4 µm thickness were made every 400 µm at femoral, middle and tibial levels by use of REM-700(YAMATO KOHKI INDUSTRIAL CO., LTD., JAPAN). They were de-paraffinized in xylene after mounting on glass slides and stained with hematoxylin and eosin. Measurements of the total ligamentous area, the fibrous one, the synovial one and the blood capillary one, they were made using Image-J 1.51n (U.S. National Institutes of Health, Bethesda, MD, USA). The number of capillary cross sections was also counted at each level.

Statistical analysis. The obtained histological data were analyzed by Mann-Whitney U-test with Bonferroni's p-value adjustment using SPSS 28.0 (IBM SPSS Statistics 28.0.1 Authorized). $P < 0.05$ was considered to be statistically significant.

RESULTS

Gross anatomical observation. The superficial layer of the knee joint injected with dye resin showed that the middle genicular artery branching from the popliteal artery runs toward the ACL. The branching inferior genicular artery crawled along the inner and outer superficial layers of the knee joint ventrally, turned toward the subpopliteal fat body and ran toward the ACL (Fig. 1).

Histological observation. Cross sections of the ACL fiber bundles were relatively small and sparse at the femoral level and became bigger and denser toward the middle and tibial levels (Fig. 2). In particular, at the femoral level, the synovium not only covered the surface of ACL but also abundantly penetrated deeply between the fiber bundles of the ACL, and the intra-synovial capillaries were well observed (Fig. 3). In contrast, at the middle level, penetration of the synovium into the fiber bundles was poor and intra-synovial capillaries were seemed apparently few. At the tibial level, the synovial penetration into the fiber bundles and distribution of intra-synovial capillaries were observed like at the femoral level but the capillaries seemed poor than those at the femoral level (Fig. 4).

Statistical analyses of blood supply to the ACL. Cross-sectional areas of the fibrous, synovial, and vascular components of the ACL were statistically compared among the femoral, middle, and tibial levels using 14 ACL samples.

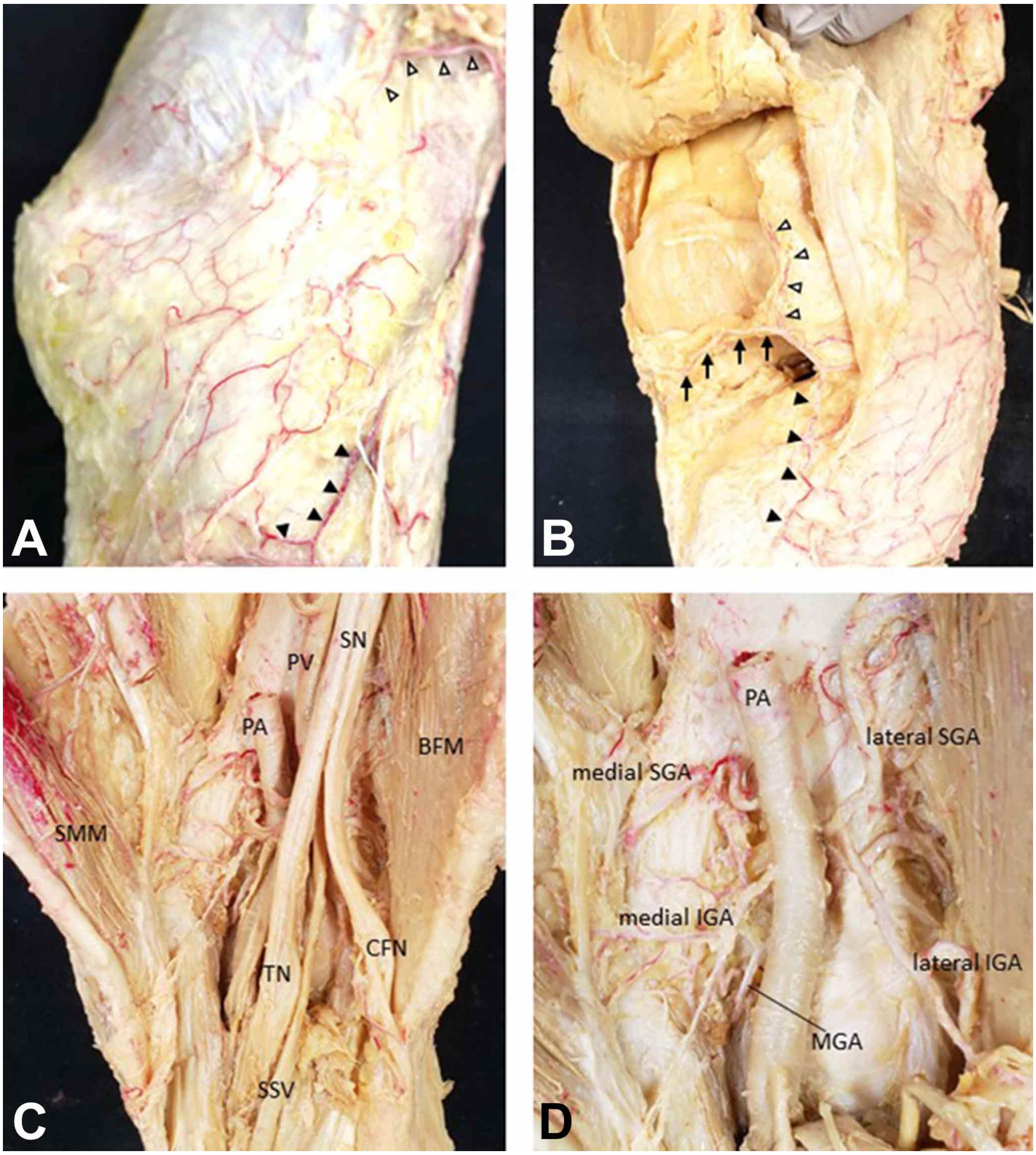

Fig. 1. Blood vessels containing dye resin that was injected from the femoral and popliteal arteries in a cadaver. A. A medial view of the right knee. The branching superior and inferior genicular arteries were observed to crawl along the inner and outer superficial layers of the knee joint ventrally. B. A frontal view of the right knee. The patellar ligament was cut and flipped outward. A blood vessel was seen running transversely anterior to the ACL toward the subpopliteal fat body from a common branch of superior-inferior genicular arteries. C. A dorsal view of the right knee. The subpopliteal fat body was removed after the biceps femoris and semimembranosus muscles were flipped to the outside. D. A dorsal view of the right knee. The popliteal vein and sciatic nerve were removed. The middle genicular artery branching from the popliteal artery ran toward the posterior intercondylar fossa. The medial and lateral superior-inferior genicular arteries ran around to the ventral side. Arrows: a common branch of superior-inferior genicular arteries, white arrow heads: a branch of superior genicular artery, black arrow heads: a branch of inferior genicular artery, BFM: biceps femoris muscle, CFN: common fibular nerve, IGA: inferior genicular artery, MGA: middle genicular artery, PA: popliteal artery, PV: popliteal vein, SGA: superior genicular artery, SMM: semimembranosus muscle, SN: sciatic nerve, SSV: small saphenous vein, TN: tibial nerve

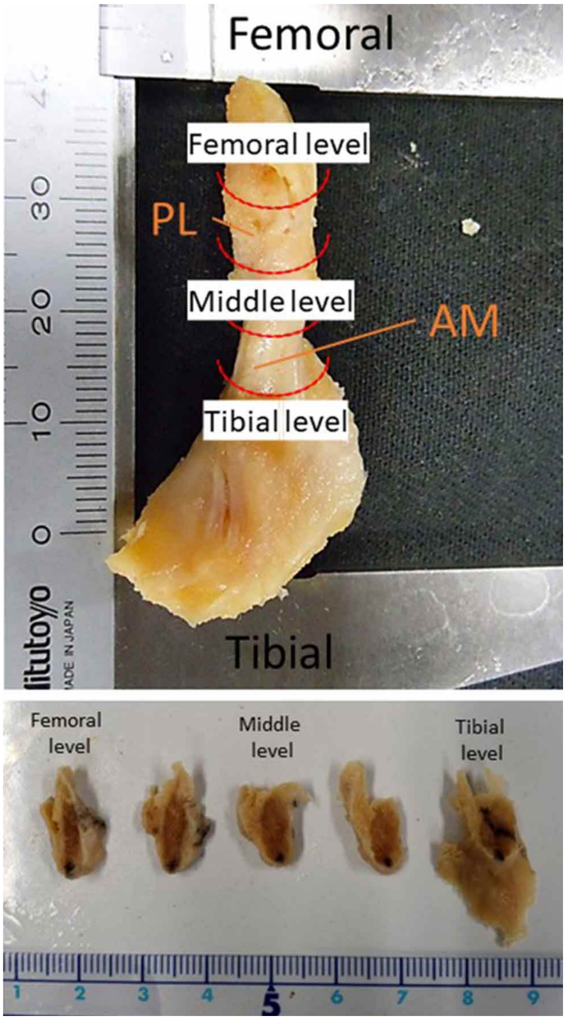

Fig. 2. Gross anatomical views of ACL and its cross-sections. A. The solid red line indicates sectional lines of the 5 equal division of ACL. B. Five cross sections of ACL, AM: anteromedial bundle, PL: posterolateral bundle.

Absolute cross-sectional ACL area at the femoral, the middle and the tibial levels

Total ligament area. Cross-sectional whole ligament area at the femoral, the middle and the tibial levels were 18.42±0.59 mm^2, 45.64±1.82 mm^2, and 52.22±1.32 mm^2, respectively. The area at the femoral level was apparently smaller than that at the middle or the tibial levels ($P < 0.01$). There was no significant difference between the middle and the tibial levels (Fig. 5a).

Fiber area. Cross-sectional fiber area at the femoral, the middle and the tibial levels were 10.72 ± 0.45 mm^2, 36.04 ± 1.60 mm^2, and 41.77 ± 1.03 mm^2, respectively, showing similar tendency obtained in whole ligament area. The area of at the femoral level was apparently smaller than the middle and the tibial levels ($P < 0.01$). There was no significant difference between at the middle and the tibial levels (Fig. 5b).

Synovium area. Cross-sectional synovium area at the femoral, the middle and the tibial levels were 7.70±0.24 mm^2, 9.61±0.72 mm^2, and 10.46±0.33 mm^2, respectively. The synovium area at the tibial level was significantly higher than that at the femoral level. However, there was no significant difference between the femoral and the middle levels and also between the middle and the tibial levels (Fig. 5c).

Blood capillary area. In general, nutritional supply to the ligament is from many capillaries in the adjacent synovium. Actually, many capillaries were identified within the synovium but the capillaries were hardly seen in the fiber structures at the light microscopical level. Cross-sectional blood capillary area at the femoral, the middle and the tibial levels were 1.24 ± 0.07 mm^2, 0.35 ± 0.01 mm^2, and 0.78 ± 0.06 mm^2, respectively (Fig. 5d). The ratio at the femoral level showed significantly higher than that at the middle level ($P < 0.01$). And also, the ratio at the

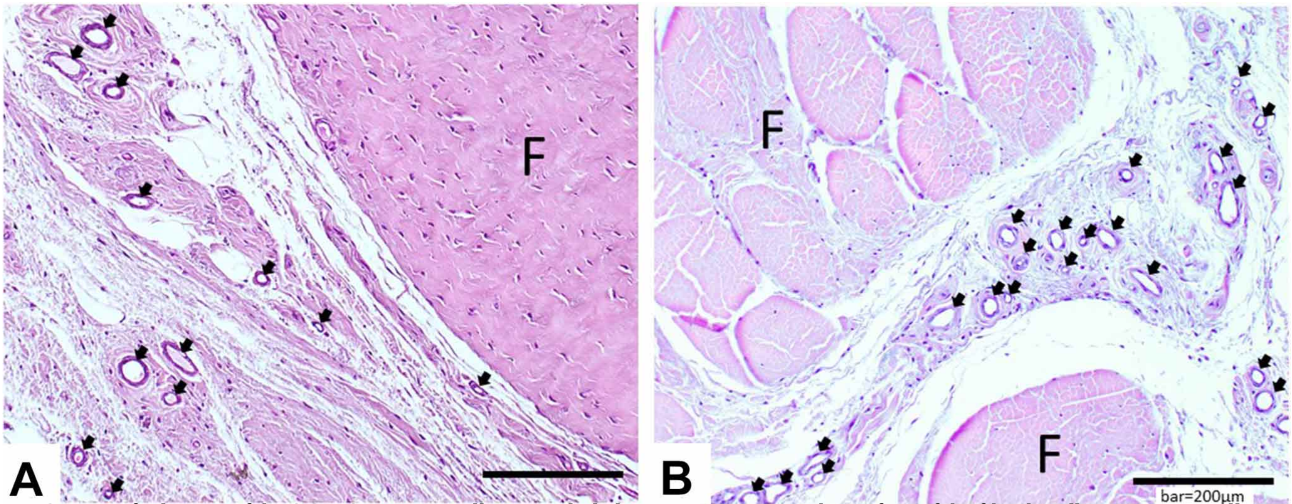

Fig. 3. Magnified views of the ACL. A. Many capillaries-included synovium covering the surface of the fiber bundle. B. Many capillaries-included synovium penetrating deeply between of the fiber bundle. Arrow: blood capillary, F: fiber bundle

tibial level showed significantly higher than that at the middle level (P < 0.05). There was no significant difference between the femoral and the tibial levels. Therefore, although cross-sectional area of ACL at the femoral level was most small among the three levels, its blood capillary area was significantly large, showing that ACL at the femoral level is rich in blood microcirculation. Indeed, it was found that the number of sectioned capillaries at the femoral, the middle and the tibial levels are 327.90±17.75, 104.95±4.76 and 204.18±10.52, respectively (P < 0.01, femoral vs middle), (P<0.05, tibial vs middle).

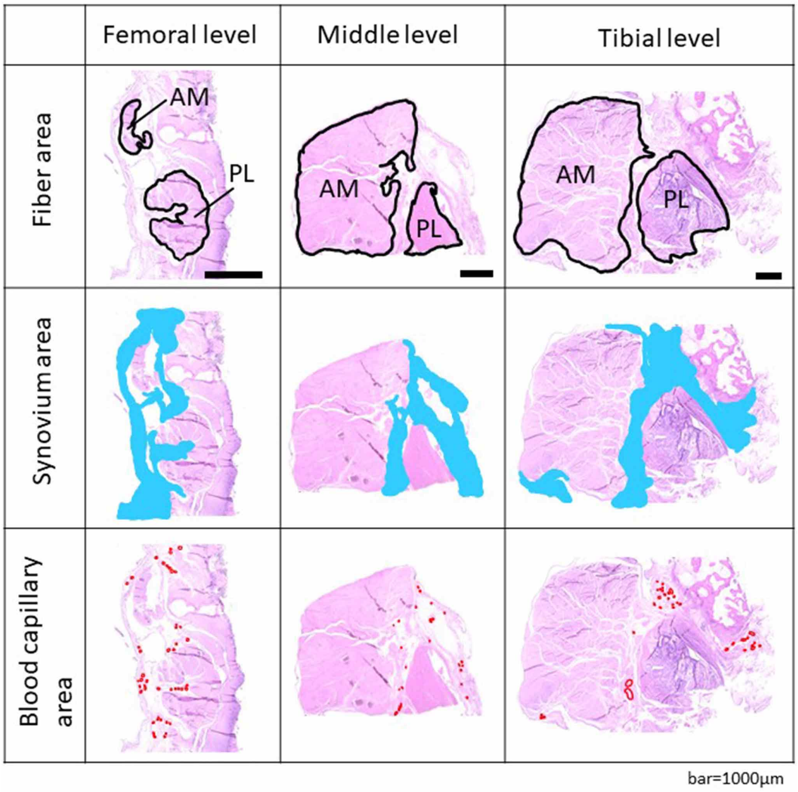

Fig. 4. Histological observation of ACL cross sections at the femoral, the middle and the tibial levels. The fiber area was circled by black line. The synovium area was shown by blue area. The blood capillary area was pointed by red marks.

Relative cross-sectional ACL area at the femoral, the middle and the tibial levels

Fiber area/Total ligament area. The ratio at the femoral level showed significantly lower than that at the middle and the tibial levels (P < 0.01). There was no significant difference between the middle and the tibial levels (Fig. 6a).

Synovium area/Total ligament area. The ratio at the femoral level showed remarkably higher than that at the middle and tibial levels (P < 0.01). There was no significant difference between the middle and the tibial levels (Fig. 6b).

Blood capillary area/Total ligament area. The ratio at the femoral level was quite high among the three levels. There was no significant difference between the middle and the tibial levels (Fig. 6c).

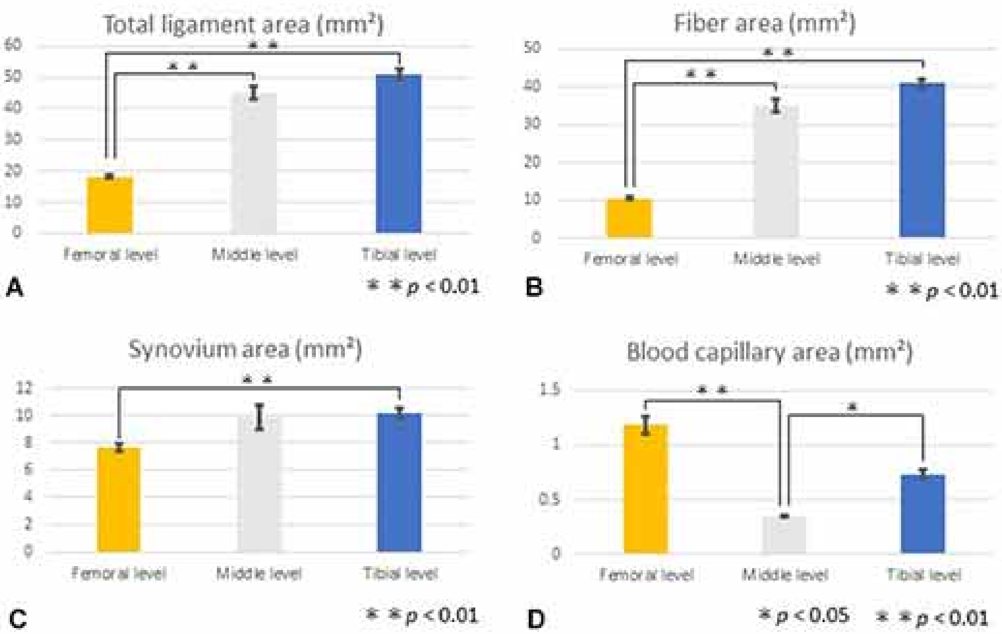

Fig. 5. Absolute cross-sectional area of ACL and its components at the femoral, the middle and the tibial levels.
p<0.05, p<0.01.

Blood capillary area/Synovium area. It was particularly noted that the ratio at the femoral level was significantly higher than that at the middle and at the tibial levels. It showed that not only total blood capillary area but also its density in the synovium was remarkably high at the femoral level. There was no significant difference between at the middle and at the tibial level (Fig. 6d).

DISCUSSION

The most important findings in the present study were that although cross-sectional ACL area at the femoral level was relatively small, the total blood capillary area was significantly larger than that at the tibial level. Furthermore, the density of the blood capillary in the synovium at the femoral level was also significantly higher than that at the tibial level. This indicates that the blood microcirculation at the femoral level is more abundant than that at the tibial level. Therefore, it became statistically evident for the first time to demonstrate the presence of more dense capillaries at the femoral level compared with the tibial level. To our best knowledge, there is only one study documenting the same findings (Petersen & Tillmann, 1999), however, in that study, there is no demonstration of morphological data and the related finding was just mentioned in the discussion but not the results of the article.

In ACL reconstruction, the tibial remnant has been often preserved, however, the femoral remnant tends to be totally resected to preserve the visual field and prevent bone tunnel malposition at the time of bone tunnel creation. ACL reconstruction with preserving tibial remnant is a simple procedure and drilling entirely within the ACL tibial stump using a remnant-preserving reconstruction technique does not significantly change the rate of tunnel malposition determined by postoperative 3D-CT when compared with stump ablation and utilization of standard landmarks (de Padua *et al*., 2021). The synovium and ligamentous tissues of the femoral attachment are directly supplied with blood from the middle genicular artery. In particular, we found that the femoral attachment rather than the tibial attachment has a dense and large distributions of blood capillaries. Therefore, ACL reconstruction with preserving femoral remnant may promote the following blood supply to the graft for its remodeling.

Not only recovery of muscle strength and motor function but also graft remodeling is essential for early return to work after ACL reconstruction (Terauchi *et al*., 2016). Lee *et al*. (2006) reported that ACL reconstruction with preserving of both femoral and tibial remnants can achieve graft remodeling at an early stage, and that the graft can be remodeled in a shorter period of time than ACL reconstruction with no remnants. Zhang *et al*. (2014) reported that ACL reconstruction with preserved remnant on the tibial

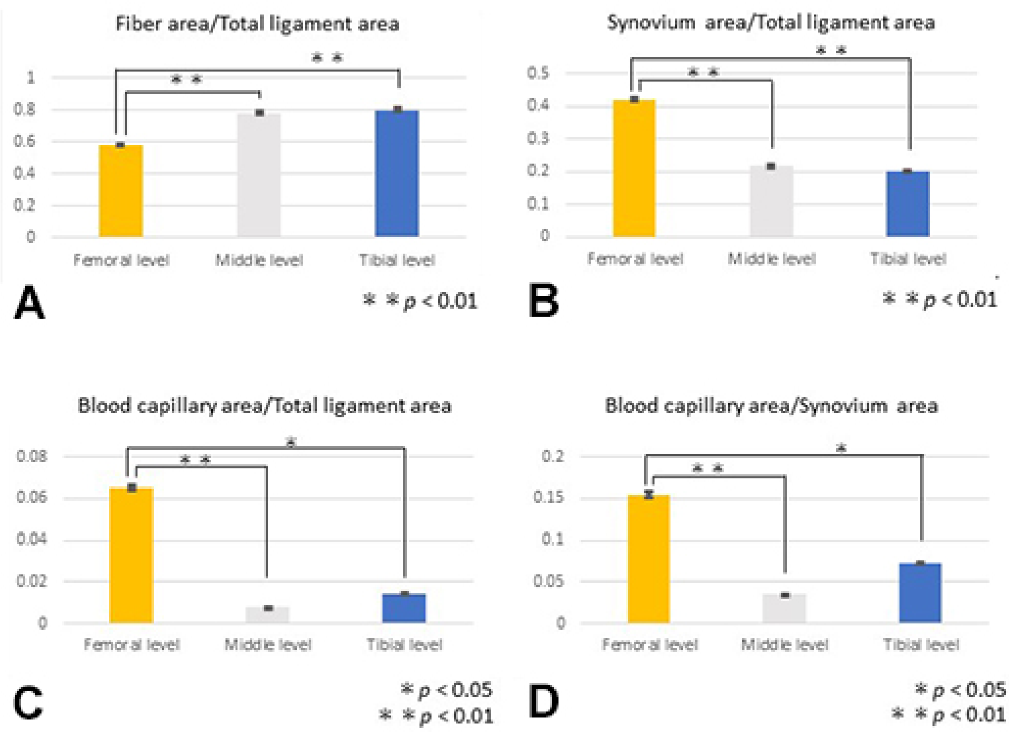

Fig. 6. The relative ratio of cross-sectional ACL area and its components at the femoral, the middle and the tibial levels. p<0.05, p<0.01.

side can resist tibial tunnel enlargement. To our knowledge, there are many reports of the usefulness with preservation of the tibial remnant, but few reports of the usefulness of preservation of the femoral remnant. The reason the tibial but not the femoral remnant is preserved, may be due to a difficulty to preserve the visual field and prevent bone tunnel malposition at the time of femoral bone tunnel creation.

Considering that the femoral remnant is more vascularized than the tibial remnant, preserving femoral remnant may be more advantageous for graft revascularization and remodeling than the tibial remnant. As ACL reconstruction techniques using highly accurate navigation systems and robot-assisted technology become standardized, accurate bone tunnel creation with minimal clearance of the femoral remnant will be possible (Cho *et al*., 2018; Guo *et al*., 2020; Liu *et al*., 2020). By the use of the newly-developed navigation systems, the revascularization and remodeling of the graft can be more easily possible, and early return to work after surgery can be expected by preserving the femoral remnant.

CONCLUSION

We examined histological cross-sectional images of the ACL at the femoral attachment, the tibial attachment and its middle level by using cadavers. The results showed that the cross-sectional ACL area at the femoral attachment is significantly small, however, the blood capillaries were most abundant. Therefore, when the ACL is injured, its reconstruction with preservation of the femoral ligamentous remnant may be clinically useful for effective remodeling of the grafted tendon.

Availability of data and materials. The data used to support the findings of this study are available from the corresponding author on reasonable request.

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RESUMEN: El ligamento cruzado anterior (LCA) es un ligamento que controla principalmente la movilidad anterior y rotacional de la articulación de la rodilla, y su superficie está cubierta por una membrana sinovial con gran cantidad de vasos sanguíneos. En general, el suministro de nutrientes al ligamento proviene de muchos capilares en la sinovial adyacente. Sin embargo, los estudios estadísticos de los capilares distribuidos en el LCA son insuficientes. En este estudio, examinamos imágenes histológicas transversales de la inserción femoral (nivel femoral), el nivel medio del tendón (nivel medio) y la inserción tibial (nivel tibial) del LCA y analizamos estadísticamente la distribución de los capilares sanguíneos entre los tres niveles. Los LCA de 10 cadáveres se dividieron en 5 secciones iguales y se realizaron cortes en parafina de 4 µm de espesor a nivel femoral, medio y tibial, y luego se realizó tinción con hematoxilina-eosina (HE). El área de cada sección transversal se midió utilizando Image-J 1.51n (Institutos Nacionales de Salud de EE. UU., Bethesda, MD, EE. UU.). Los haces de fibras del LCA eran relativamente pequeños y escasos en el área de la sección transversal a nivel femoral y se hicieron más grandes y más densos hacia el nivel tibial. La membrana sinovial en la unión del LCA cubría la superficie del haz de fibras y también penetraba profundamente entre entre los haces de fibras. En particular, los capilares sanguíneos estaban densamente distribuidos en la unión femoral de la sinovial respecto a los otros dos niveles. De hecho, el número de capilares también fue más abundante a nivel femoral. El área transversal del LCA a nivel femoral era significativamente pequeña, sin embargo, los capilares sanguíneos fueron los más abundantes. Por lo tanto, cuando hay una lesión del LCA su reconstrucción con preservación del ligamento femoral remanente puede ser clínicamente útil para remodelar el tendón injertado.

PALABRAS CLAVE: Ligamento cruzado anterior; Capilar sanguíneo; Sinovial; Reconstrucción del LCA; Remodelación.

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