Morphometric Study of Anterior Cruciate Ligament and Histological Comparison with the Patellar and Hamstring Tendons and a Unique Case of Pes Anserinus Variant

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Abstract: Tendons are being used as grafts for the ACL reconstruction. Their microscopic structure has not been sufficiently studied and compared to the ACL. Aim: The present study focused on the anatomy of the anterior cruciate ligament as well as microscopic structure of patellar tendon, semitendinosus and gracilis tendons. It investigated also the crimping patterns and the tensile strength of these tendons and ligaments. Methods. length and width of ACL were measured by MRI software program for 15 patients. Twenty six lower limbs from formalin fixed cadavers were used in the present study. Measurement of tibial and femoral attachment of ACL were determined with the help of digital caliper. Samples from four different human tendons (ACL, Patellar tendon, semitendinosus and gracilis)were studied by light and scanning electron microscopy. The number, angle and length of wave crimps of the collagen fibers were analyzed by scanning electron microscopy and fibril/interstitum ratio and density of fibroblasts were also analyzed. Results. The length of the anteromedial and posterolateral bundles were 32±3.5mm and 23±3mm respectively with nearly the same width. The mean length and width of the femoral attachment of ACL were 17.3±1.7 mm and 9.3 ± 1.1 mm respectively, while, the mean length and width of the tibial attachment were 18.3 ± 2.9 mm and 12.1 ± 2.8 mm respectively. The crimp angle of the collagen fibers varied greatly between the four different tendons. Crimp top angle in ACL and PT (88 ± 8.6º and 130 ± 9.7 º respectively) was significantly lower than in semitendinosus and gracilis (148 ± 10 º and 159 ± 10.5 º respectively). Morphometric analysis confirmed that crimp number was highest in ACL and PT (6.2 ± 0.7and 5.4 ± 0.6 respectively). The crimp base length was more longer in gracilis (22 ± 4.1µm) and semitendinosus (20 ± 4.1 µm) than in ACL (15 ± 3.2 µm). The semitendinosus showed the highest number of fibroblasts, while the gracilis showed the highest fibril/interstitium ratio. No significant differences regarding the diameter of the collagen fibrils were found between all groups. The tensile strength was highest in PL (2905 ± 127 N) compared to other groups. A unique case of pes anserinus variant is reported that had implications for tendon harvesting and have an impact on the outcome of surgery. CONCLUSIONS: The present study will be useful for enhancing the knowledge of anatomy of ACL. Both semitendinous and gracilis tendons provide significantly more density of collagen fibrils and fibroblasts in comparison with patellar tendons. These findings provide a potential advantage of the hamstrings group on better remodelling and regeneration of the tissue.

[Mohamed Atif Ahmed Said Ahmed. **Morphometric Study of Anterior Cruciate Ligament and Histological Comparison with the Patellar and Hamstring Tendons and a Unique Case of Pes Anserinus Variant.** *Life Sci J* 2013;10(1):1402-1411] (ISSN:1097-8135). http://www.lifesciencesite.com. 210

Keywords: anterior cruciate ligament – patellar tendon – semitendinosus – gracilis – pes anserinus variant.

1. Introduction

The integrity of the anterior cruciate ligament (ACL) is important to athletes who participate in running and jumping sports.[1] The incidence of ACL ruptures has risen during the last years. Currently, a large number of ACL reconstructions are performed each year.[2] To achieve a satisfying surgical outcome after ACL reconstruction, a basic knowledge of the anatomy of the ACL is essential. The ACL origin is from the medial surface of the lateral femoral condyle to be inserted into an area on the tibia between the medial intercondylar eminence and the anterior horns of the menisci.[3] Length and diameter of the ACL play an important role for selection and preparation of the graft. Little is known about the length and diameter of the ACL. There is also disagreement on the actual anatomic division of the ACL.[4,5,6]

Currently, most ACL reconstructions are performed with patellar tendon or hamstrings autograft. However, there has been recent interest in the use of allograft.[7]

Ligaments and tendons have often been considered as similar tissues with no marked difference due to their similar composition and role in transmitting mechanical forces. [8,9] Others suggest that they may be structurally different from each other so that one tendon is not like another.[10,11] The histological structure of the tendons used as grafts for the ACL reconstruction has not been sufficiently studied in the human being and compared to the ACL.[12]

The material properties of ligament and tendon published in the literature vary considerably. Tensile tests are commonly used to characterize the structural and material properties of tendons and ligaments.[13] These tests provide important data on the function of tissues and the mechanisms of injury and healing.[10,14] Graft tension can aid clinicians in graft selection and is an important factor in achieving a successful outcome after ACL reconstruction.[15]

The pes anserinus is composed of a combination of tendinous insertions of the sartorius (SA), gracilis (GL) and semitendinosus (ST) muscles.[16] Precise anatomical knowledge of the structures on the medial side of the knee and the relationships between tendons is critical for diagnosis, surgery, and the development of improved operative procedures of the knee.

The SA is the longest muscle in the body. It is used as a reconstructive flap for the abdominal wall, the inguinal zone and the distal third of the thigh.[17,18] The ST tendon curves around the medial condyle of the tibia to be inserted into the upper part of the medial surface of the body of the tibia with the GL muscle at the Gerdy tubercle behind the tendon of the ST, where it contributes to the pes anserinus.[3] The semimembranosus muscle (SM) is inserted mainly into the horizontal groove on the posterior medial aspect of the medial condyle of the tibia.[3]

The ST and GL tendon are commonly used for ligament reconstruction. Inaccurate incision may cause complications such as cutting the main tendon, inadequate graft length and tibial nerve injury.[19] The harvesting of ST and GL is still met with certain anxiety by many surgeons, as it is a closed, blind procedure. Pes anserinus variations may predispose certain patients to injury that may lead to a decrease in the normal glide and flexibility of the muscles.[20] The variant of the pes anserinus is dangerous as it can potentially divert the course of the tendon stripper and divide the main tendon resulting in a short inadequate graft. Little has been reported concerning the anatomy of these variant.

This study will be focused on the anatomy of the ACL as well as microscopic structure of the ACL, PT, ST and GL tendons. It will also investigate the crimping patterns and the tensile strength of these tendons and ligaments. Unique case of a variant of the pes anserinus and semimemranosus muscle will be discussed.

2. Material and Methods

Magnetic resonance images of the knee were prospectively collected as part of routine diagnostic workups for 26 consecutive adult patients visiting the department of radiology, Faculty of Medical Sciences, King Khaled University. The knee positioned in full extension and the images were collected using 6-mm section thickness by (General Electric Machine). Standard sagittal and coronal images were used. Eleven patients were excluded due to ACL pathology leaving a total of 15 images for review. Ten males and five females with mean age (41.6 ± 6.5 years). Basic data were collected for each patient.

The intact ACL was measured. The images were independently measured in a blinded fashion by 2 specialists. The measurements were obtained by using the ruler function on a digital radiology viewing program. The average length and width of the bundles were calculated for each examiner and for both examiners combined.

The anatomical and histological parts of the present study was conducted in the Anatomy Department at King Khaled University, College of Medicine. Twenty six human cadaveric lower limbs were studied. There were 8 males and 5 females, with a mean age of (59.6 ± 8.1 years). No trauma or any other pathological condition was recorded in the joints studied. A careful dissection of the knee joint was made and the cruciate ligament was explored. The morphometric characteristics of both tibial and femoral attachments of the ACL were measured by digital caliber.

During the present study a careful deep dissection of the ST, SA and GL tendons was made starting from the insertion of the pes anserinus and extending proximally to the musculo-tendinous junction. All tendons of ST, GL and SA were carefully identified and their insertions at the pes anserinus were recorded.

Data were analyzed using Statistical Product and Service Solutions (SPSS). The mean was calculated with the standard of deviation. Differences between examiners were evaluated with a paired t-test.

Light microscopy

Specimens including the ACL, PT, ST and GL tendons were fixed in 10% neutral buffered formalin for 24 h, decalcified in 5% nitric acid for 5hours, dehydrated in graded alcohols and embedded in paraffin. Longitudinal sections of 10 μm were cut and then stained with Masson's trichrome to enhance the collagen under the light microscope (Nikon Eclipse E-200). These sections were used for comparative analysis of tissue structure.

The technique of point-counting morphometry was used to assess the ratio of fibril to interstatium. A sampling grid of points was used.[21] Four randomly selected fields per field were counted on each micrograph.[22] Assessment was made of whether each point was positioned on a fibril or interstatium.

The ratio of fibrils to interstatium was calculated for the tendons in each group.

SEM

The specimens for scanning electron microscopy were prepared as described previously.[23] Briefly, the specimens for scanning electron microscopy were fixed in 2% glutaraldehyde buffered with cacodylate, pH (7.4), dehydrated in graded alcohols and coated with gold. These specimens were examined and photographed with a 6390 LV Jeol SEM scanning electron microscope, College of Medicine, King Khaled University. Photomicrographs were obtained from randomly chosen areas.

Morphometry

Crimp number, crimp top angle, the corresponding crimp base length and the diameter of collagen fibrils were measured from the images obtained by scanning electron microscopy. Microscopic images obtained from each tendon or ligament were randomly chosen for analysis. For each image the total number of crimps, the angle width at the top of three randomly selected crimps and the corresponding base lengths were recorded. An ANOVA test was used to evaluate for a difference among the four groups. All analyses were carried out using Prism program for Windows. Differences were considered to be significant at $P < 0.05$. Values were means ± S.D.

The fibril/interstitum ratio and density of fibroblasts per field were recorded from the light microscopy trichrome slides and analyzed.

Tensile strength

Sixteen tendons of ACL, PT, ST and GL, four tendons per each group, were harvested from four human cadavers. Samples were cut transverse to the tendon long-axis from the middle part of the tendon. The specimens were wrapped in moistened gauze (0.9% saline). Samples were fixed to the device. The specimens were used for immediate ultimate failure loading. Failure loading was obtained with uniaxial loading parallel to the fiber axis at a deformation rate of 1 mm/s. A universal testing machine (Wp 300.20-PC measurement data recording system) (Fig. 1) was used at College of Engineering, King Khaled University, Abha, KSA. Ultimate tensile strength was recorded for each specimen according to Noyes *et al*.[24] Each measurement was performed by the same technician and repeated three times.

3. Results

The ACL, by MRI study, is divided into 2 parallel bundles, namely the anteromedial (AM) and posterolateral (PL) bundle (Fig.2). The length of the AM bundle is 32 ± 3.5mm and the width is 6.9 ± 0.9 mm. The length of the PL bundle is 23 ± 3mm and the width is 6.9 ± 0.9 mm. The width of the two bundles of the ACL bundles were the same. The width was measured at the upper, middle and lower thirds of the ligament. The middle third of the ligament was the most narrow portion.

The ACL ligament, in cadaveric study, is attached to the femur over an oval area with a mean length of 17.3 ± 1.7 mm and a width of 9.3 ± 1.1 mm with mean surface area of 130 ± 12.6 mm^2. The insertion site for the ACL on the tibia is also oval area covering a mean surface area of 148 ± 17.1 mm^2, 18.3 ± 2.9 mm length and 12.1 ± 2.8 mm width.

The ACL consists of collagen fiber bundles which forms multiple fascicles separated by septa of loose connective tissue. A bunch of collagen fibrils forms a collagen fiber, which is the basic unit of a tendon. Collagen fibers showed a longitudinal arrangement along the ligaments. Some of the individual fibrils and fibril groups form spiral-type plaits. Collagen fiber bundles were separated often by thin spaces. The major cell type of the cruciate ligament is the elongated fibroblast, lying solitarily between the parallel collagen fibrils and appear elongated and frequently has cytoplasmic processes. Histological sections also reveal the presence of chondrocytes-like cells (Fig. 3).

The anterior cruciate ligament by SEM showed collagen fiber bundles running parallel in a helical and planar wave pattern. ACL was appeared to be a thick multilayer cell sheet. Each layer included densely packed large collagen fibrils coursing parallel with periodic crimps (Fig. 4). Inside these tendon crimps some of the parallelly coursing fibrils changed their direction showing knots considered as fibril crimps. Tendon crimps appeared smaller, more numerous and closer to each other (Fig. 4).

PT in longitudinal sections appeared to be composed of high densely packed collagen fibrils. Collagen fiber bundles running parallel and arranged in crimps. Crimps are clearly large and flatten in longitudinal sections. Fibril crimps are also appeared. Collagen fiber bundles in the PT appear separated by large spaces in comparison to other tendons studied and the cellular material is rare (Fig. 5).

The PT at SEM showed high densely packed fibrils coursing parallel, but interrupted by irregular crimps. Tendon crimps appeared wider, less numerous and not closer to one other (Fig. 6).

ST in longitudinal sections appeared to be composed of densely packed collagen fibrils. Collagen fiber bundles running parallel and arranged in crimps. Crimps were wide in longitudinal sections. Fibril crimps are also appeared. More fibroblasts appear in the histological section and the interstitial spaces is moderate in size in comparison to other

tendons studied. The cell bodies of the fibroblasts are lodged in rows between fibers (Fig. 7).

SEM of the ST showed densely packed fibrils coursing parallel, but interrupted by large, wide, less numerous crimps (Fig. 8).

The GL tendon is composed of longitudinally aligned collagen fibrils arranged in bundles. Fibroblasts are arranged in rows between the fibers. The interstitial space is narrow (Fig. 9).

SEM of the GL showed running parallel fibers separated by thin spaces. Each fiber included densely packed fibrils coursing nearly straight, but interrupted by wide flat crimps (Fig. 10).

Table 1. Comparative analysis of morphometric data in ACL, PT, ST and GL tendons.

	Cruciate ligament	Patellar ligament	Semitendinosus tendon	Gracilis tendon
Mean number of fibroblasts/field	7 ± 0.8	1 ± 0.5	7 ± 1.1	5 ± 0.7
Fibril/interstitial ratio	70% ± 9	69% ± 9	85% ± 6	92% ± 5
Crimp number	6.2 ± 0.7	5.4 ± 0.6	3.1 ± 0.5	1.3 ± 0.5
Crimp top angle (°)	88 ± 8.6	130 ± 9.7	148 ± 10	159 ± 10.5
Crimp base length (μm)	15 ± 3.2	18 ± 3.7	20 ± 4.1	22 ± 4.1
Diameter of collagen fibril	120 ± 46 nm	127 ± 51 nm	118 ± 46 nm	119 ± 44 nm
Tensile strength (N)	1680 ± 95	2905 ± 127	1186 ± 85	980 ± 79

There is a significance differences ($P < 0.05$) between all tendons and ligament studied in all previous items except the diameter of collagen fibril. The results of the present study showed that the histological structure of the ACL differs from those of the PT, ST and GL tendons.

The GL tendon, in comparison with the ST tendon, provides a significantly higher fibril/interstitium ratio. The GL tendon has approximately 7 % more fibers than the ST tendon.

The ST and GL tendons provide more fibril/interstitum ratio and the density of fibroblasts is more compared with the PT. No significant differences found regarding the diameter of the collagen fibrils among all groups.

The ST showed the highest density of fibroblasts, while the GL the highest fibril/interstitium ratio. ACL and PT are higher in crimp frequency and the lesser is for the GL tendon.

Morphometric analysis

The crimp number is higher in ACL (6.2 ± 0.7), whereas it is 5.4 ± 0.6 in PT and less in ST and GL tendons (3.1 ± 0.5 and 1.3 ± 0.5) respectively. ACL has a significantly ($P < 0.05$) greater quantity of crimps than PT, ST and GL (Table 1).

The mean of crimp top angle in ACL and PT is 88 ± 8.6° and 130 ± 9.7° respectively, whereas it was higher in ST(148 ± 10°) and GL tendons (159 ± 10.5°). The crimp top angle is significantly ($P < 0.05$) smaller in ACL as compared to PT, ST and GL tendons. ACL shows a much smaller crimp base length (15 ± 3.2 μm) than PT (18 ± 3.7μm), ST (20 ± 4.1 μm) and GL (22 ± 4.3 μm). Crimp top angle, number and width were significantly different among the groups ($P < 0.001$).

Tensile strength

The mean tensile strength of the ACL is 1680 ± 95 N. The tensile strength of the PT is 2905 ± 127 N. ST and GL tendons have tensile load of 1186 ± 85 and 980 ± 79 N, respectively.

Unique case of pes anserinus variant

An anatomical variant of the pes anserinus encountered during cadaveric dissection in this study has not been previously described. At the distal portion, it was noted that SA tendon attached to the GL tendon by a slip of aponeurotic fascia in the upper part of the tibia. The slip from the SA tendon had attached to the GL tendon and vice versa. The tendon of SM curves anteriorly to join the tendons of the GL muscle by band of aponeurotic fascia, finally inserting into the upper part on the medial surface of the tibia. The tendons of SA and GL continuous together and passing distally to be joined with ST tendon to the anterior part of the anteromedial surface of the middle of the tibia for inserted there. SA muscle length was 64 cm. The insertion site of SA, GL and ST tendons is about 15 cm away from the normal site of pes anserinus. This variant is unique and to the best of our knowledge has not reported earlier.

4. Discussion

Some authors describe the ACL as one entity,[5] while, multiple studies have identified separate bundles of the ACL. Amis and Dawkins[6] divided the ACL into an anteromedial, intermediate, and posterolateral bundles. The anatomy of the intact ACL described in many published papers as to be composed of two bundles named according to the position of the bundle on the tibia as AM bundle and PL bundle.[4,25]

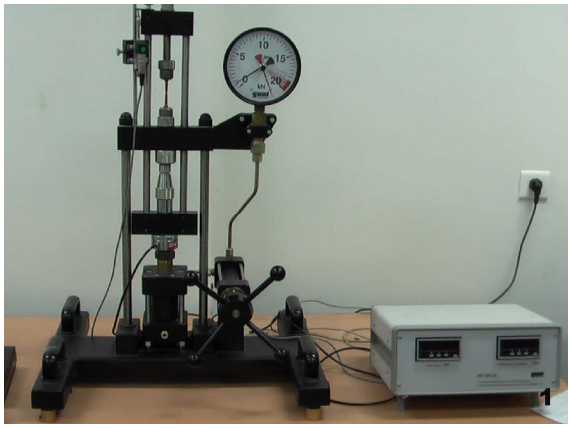

Fig. 1. Photograph of tensile strength machine (Wp 300.20- PC measurement data recording system for universal machine).

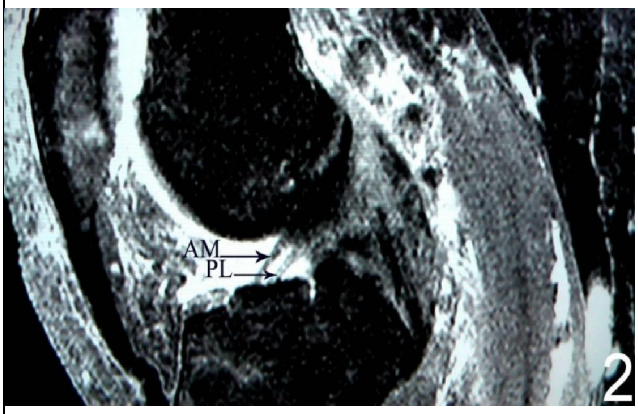

Fig. 2. MRI of Knee joint showing the anteromedial (AM) and posterolateral (PL) bundles of the anterior cruciate ligament

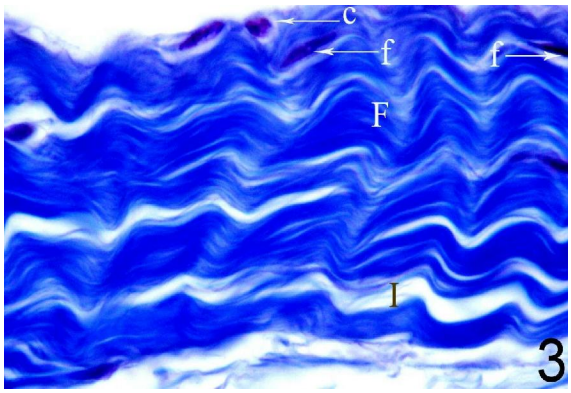

Fig. 3. A photomicrograph of section of the anterior cruciate ligament showing fibre (F) with their fibroblasts (f) and chondrocyte like cells (c). Notice the interstitial space (I) lying between the fibres. (Masson´s trichrome X 1000)

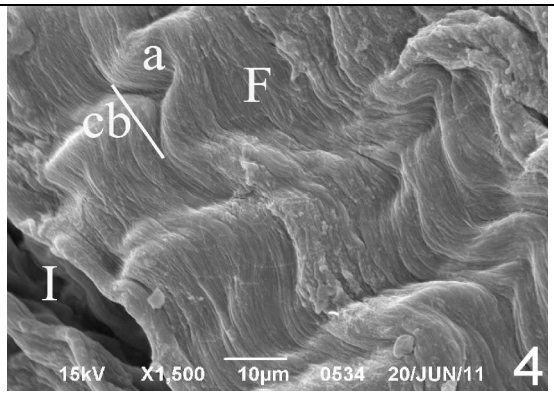

Fig. 4. SEM micrograph of the anterior cruciate ligament showing the collagen fibre bundles (F) running parallel and appear separated by thin interstitial space (I). Inside each bundle densely packed collagen fibrils run parallel showing tendon crimps. The crimb base (cb) and the crimb angle (a) are noticed. Bar = 10 µm.

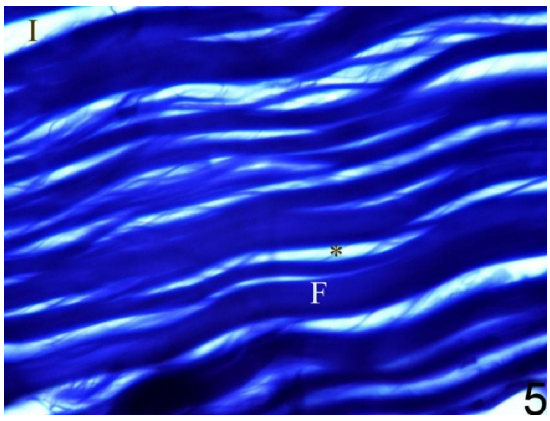

Fig. 5. A photomicrograph of a section in the patellar ligament showing high densely packed collagen fibrils. Fibre crimps appeared wider. Notice the fibre (F), the interstitial space (I) between the fibres and the fibril crimp (*). (Masson´s trichrome X 1000)

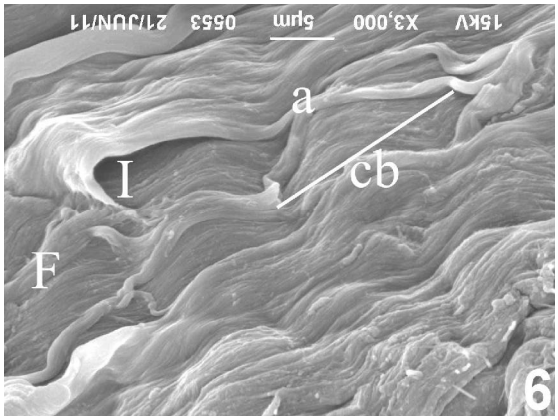

Fig. 6. SEM micrograph of the Patellar ligament showing the collagen fibre bundles (F) running parallel. Inside each bundle highly densely packed collagen fibrils run parallel showing fibre crimps. The crimp base (cb), crimp angle (a) and the wide interstitial space (I) are noticed. Bar = 5 µm.

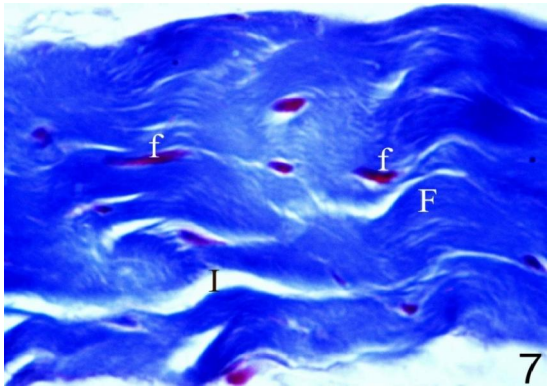

Fig. 7. A photomicrograph of a section in the semitendinosus tendon showing densely packed collagen fibrils. Notice the fibre (F) and the interstitial space (I) between the fibres. Fibre crimps appeared wider and the fibroblasts (f) are arranged in parallel rows between bundles of collagen fibrils. (Masson´s trichrome X 1000)

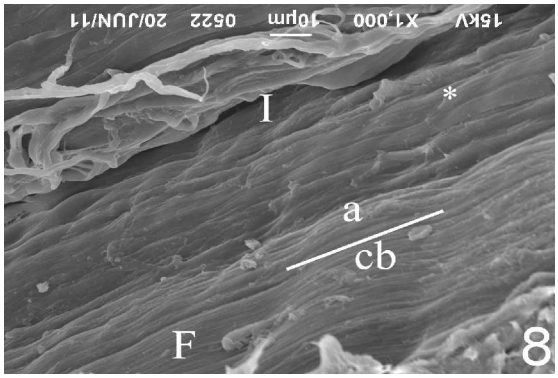

Fig. 8. SEM micrograph of the semitendinosus tendon showing the collagen fibre bundles (F) running parallel. Inside each bundle densely packed collagen fibrils run parallel showing wide tendon crimps and mild interstitial space (I). The crimp base (cb), crimp angle (a) and the fibril crimp (*) are seen. Bar = 10 µm.

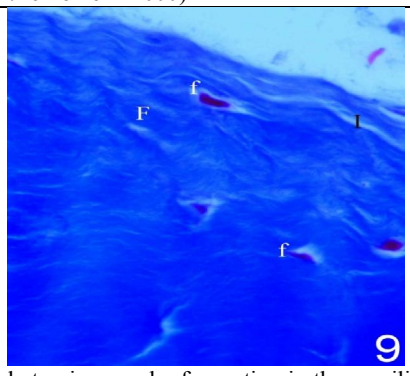

Fig. 9. A photomicrograph of a section in the gracilis tendon showing densely packed collagen fibrils. Notice the fibre (F) and the narrow interstitial space (I) between the fibres. Fibre crimps appeared more wider and the fibroblasts (f) are arranged in parallel rows between bundles of collagen fibrils. (Masson´s trichrome X 1000)

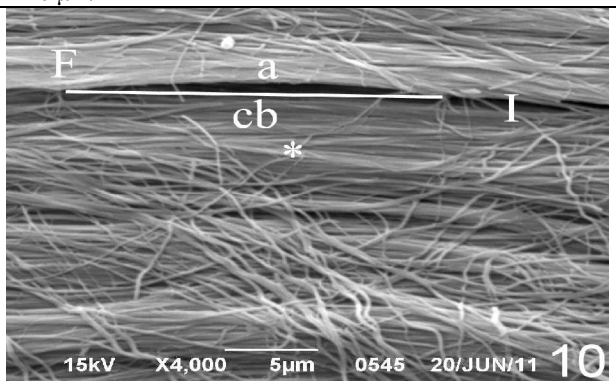

Fig. 10. SEM micrograph of the gracilis tendon showing the collagen fibre bundles (F) running parallel. Inside each bundle densely packed collagen fibrils with more wider tendon crimps. The crimp base (cb), crimp angle (a), the narrow interstitial space (I) and the fibril crimp (*) are seen. Bar = 5 µm.

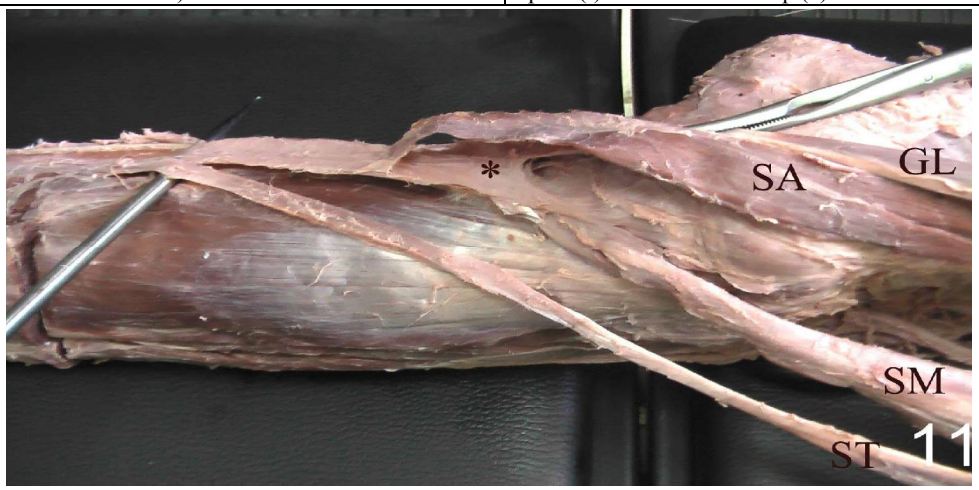

Fig. 11. A photograph of a unique case of pes anserinus variant. Notice the fascia (*) connecting the gracilis (GL), sartorius (SA) and semimembranosus (SM) tendons and the site of insertion of sartorius, gracilis, semitendinosus (ST) and semimembranosus tendons.

In agreement with the anatomical studies and recent data,[26] the present study showed that the ACL in MRI is divided into two parallel bundles, namely the AM bundle and the PL bundle. The AM bundle responsible for controlling the anteroposterior stability of the knee, and the PL bundle is responsible for controlling the internal rotation of the lateral tibial plateau.[27]

The length of the AM bundle in the present study was 32±3.5 mm and of the PL bundle was 23±3 mm by MRI. The width of each bundle is the same 6.9 ± 0.9 mm. The middle third of the ligament is the most narrow portion. The present result is in agreement with Takahashi *et al*.[28] and Smith *et al*.[29] Hollis *et al*.[30] measured the individual bundle lengths in dissected cadavers to be, on average, 34 mm for the AM bundle and 22.5 mm for the PL bundle. Similar results were found by Steckel *et al*. [31] in a cadaveric model, in which the average length and width of the AM and PL bundles were 37.7 and 8.5 mm and 20.7 and 7.7 mm, respectively. The present data obtained by MRI demonstrate nearly the same trend, in which the AM bundle was longer than the PL bundle. Several studies have shown that the length of the ACL fibers vary with motion of the knee. The distance for the AM bundle increases with flexion and the length of the PL bundle decreases with flexion.[30, 32] Length and diameter of the ACL play an important role in ACL reconstruction.

Grossly, the femoral attachment is oval in shape with surface area of 130 ± 12.6 mm^2. Authors using laser-digitizing methods described the insertion site to be circular [25] or oval[5] with a mean length of 18 mm and a width of 11 mm. The tibial attachment of ACL consists of oval area with surface area of 148 ± 17.1 mm^2, 18.3 ± 2.9 mm length and 12.1 ± 2.8 mm width. Harner *et al*.[25] found the tibial insertion of the ACL to be approximately 120% of the femoral insertion site.

Morphological differences between ligaments and tendons or among different ligaments or different tendons have not been investigated in depth.[11] Fibrils group into fibers, fibers into fiber bundles.[11] All these structures are mainly oriented in their axial direction to strongly resist tensile load.[11,33] ACL should be histological and morphologically different from the tendons used for ACL reconstruction.[12] The present study showed that the ACL has a unique and complex histological structure.

The fibers in the present study are arranged in a wavy manner. The wavy shape is likely to allow the tendon to have its spring-like behavior. Tendons working with gravity are more flattened than those working against gravity.[34] The absence of mechanical loading leads to the straightening of crimps in the same tendon.[23] For this reason the present study evaluated crimp number, crimp top angle and crimp base length in ACL, PT, ST and GL tendons to understand their elastic recoil properties and mechanical functions. Morphological observations and statistically significant morphometric analysis demonstrated that crimp number was high in ACL and PT, but it was less in GL and ST tendons. The mean crimp top angle in GL and ST tendons was significantly higher than in ACL and PT. The crimp base length was longer in GL and ST tendons than in ACL and PT. The smallest base length, top angle and most crimped pattern in ACL vs. PT, ST and GL tendons. Crimps in GL and ST tendons are larger, flatter and fewer than in ACL and PT suggesting ST and GL tendons acts as the major agonistic. The structure and crimping pattern observed in ST tendon were nearly similar to those of GL tendon suggesting that these tissues may have a similar recoiling property and biomechanical function. Crimps are involved in transferring and absorbing forces and recoiling of tendons and ligaments.[11] There is a correlation between the collagen fiber angle distribution and tendon mechanics in response to tensile loading.[35]

Collagen fibrils show crimps in the tendons and ligaments.[36] The single collagen fibrils within each tendon crimp change their direction showing fibril crimps.[37] The fibril crimps correspond to particular molecular arrangements of fibrils. They act as an elastic recoiling complex when a stretching force is removed.[11,33] The presence of fibril crimps in each collagen fibril inside crimps indicates a higher elastic recoil property and mechanical strength.

The cell bodies of the fibroblasts were lodged in rows between fibers and appeared elongated and frequently had cytoplasmic processes. Fibroblastic cells in tensile load have cytoplasmic processes which may be long and extend through collagen fibers.[38] The fibroblasts maintain the extracellular matrix.[39] The tendon fibroblasts proliferated faster than the ACL fibroblasts regardless of the material and geometry.[40] Myofibroblasts has been identified in fibroblastic cells and they might be involved in crimp formation and transmit tensile forces to the extracellular matrix in normal tendon and ligament.[41] Histological sections of ACL reveal the presence of chondrocytes-like cells. The presence of chondrocytes considered as a functional adaptation of the ligament to the occurring compressive stress .[42]

Fibril diameter is nearly the same in ACL, PT, ST and GL. The difference in tensile strength is not associated with fibril diameter, but with increase in collagen fibril lengths,[43] which can be related to the viscoelastic properties of tendons.[44] Other investigation has shown that collagen fibril diameter distribution alone cannot predict the material and

structural properties of a tendon.[8] Mechanical properties, soft tissue healing, cell proliferation, matrix production, and differentiation are regulated by changes in the fiber diameter.[45]

PT is widely considered a tendon. The biochemical extracellular matrix composition of human PT differs from that of other knee ligaments and is similar to other tendons.[46] All investigated tendons, excluding the patellar tendon, showed a higher fibril/interstitium ratio in comparison with ACL. A high percentage of the collagen fibrils in tendons can be important for the biomechanical capacity and the strength of tendon grafts.[47] Both ST and GL tendons provide significantly more density of collagen fibrils as well as density of fibroblasts in comparison with patellar tendons. These findings provide a potential advantage of the hamstrings group on better remodeling and regeneration of the tissue. The GL tendon showed the highest concentration of fibril/interstitium ratio. The GL tendon has approximately 7 % more fibers than the ST tendon. More fibers can play an important role for better biomechanical stability of the GL tendon. Hadjicostas *et al*.[12] reported that ligaments and tendons are similar in composition but differ in proportion and arrangement.

Tensile tests have been used to quantify the material properties of ligaments and tendons.[13,48] The tensile strength of the ACL, PT, ST, GL in the present research are 1680±95 N, 2905±127 N, 1186±85 N, 980±79 N, respectively. PT is the only ligament of the study that exceed ACL tensile strength value. The PT tensile strength is 173% greater than the ACL. The tensile strength of the ST represents 70% of the ACL tensile strength. The functional anatomy and biomechanics of the ST, GL and PT tendons explains the difference in their tensile strength. The PT functionally differs from the other knee ligaments as its load-related properties are significantly greater than the corresponding values of the ACL.[49] Younger specimens exhibited greater tensile load.[50]

Noyes *et al* reported the mean tensile strength of the normal ACL to be 1725 N.[24] The tensile strength of the ACL has been reported to be up to 2195 N.[51] Various investigation have demonstrated the tensile strength of the patellar tendon graft to be stronger than the original ACL.[52] Whereas, hamstring tendon grafts have been found to be inferior in strength to the normal ACL.[52]

The ST show the highest density of fibroblasts, while the GL the highest fibril/interstitium ratio. The highest fibroblasts and fibrils can play an important role in the better strength of the ST and GL tendons. The semitendinosus/gracilis combination is said to be stronger than the original ACL.[51,52] The technique of coupling/doubling the semitendinosus and gracilis tendons is a more commonly established procedure.[53]

It is rarity in finding variations of the pes anserinus. Ivey and Prud'homme[54] classified the anatomical variants of the pes anserinus into seven groups: 1- Semicircular fascial slips extending from the first layer to the posterior aspect of the medial collateral ligament. 2- Splitting of the ST tendon with one end joining the GL and the other joining the crural fascia. 3- Overlap between the tendons in their distal attachment. 4- Thickened SA fascia inserting either as a separate tendon, or joining the GL or ST. 5- Tendinous slips arising from SA or GL running perpendicular and superficial to the ST. 6- A thickened band of SM running parallel to the medial collateral ligament adherent to the GL and ST. 7- A slip of ST splitting prior to fusion with the GL and inserting directly into the medial collateral ligament or tibia.

Koulouris and Connell[20] reported have that the SM muscle has multiple insertions. One inserted along the posterior aspect of the lateral condyle of the femur, the posterior joint capsule and the arcuate ligament. A second is attached to the fascia which covers the popliteus muscle and the posterior oblique ligament; while a few fibers inserted to the medial tibial condyle.[55] The SM muscle may be doubled and giving a slip to the femur or adductor magnus muscle.[56,57] An accessory SM has also been described.[56] A common accessory insertion of ST to the gastrocnemius fascia and a fascial sling have been found that commonly invests ST as it emerges under the SM tendon.[3]

Slips between the hamstring muscles may result in variations in the extent of insertion sites, causing a decrease in flexibility.[20] The insertion of SM to the upper medial part of the tibia and the slip between it, and SA and GL in the present study is rare and not reported before.

SA muscle length in the presenting case is 64 cm. This measurement is comparable to those published earlier.[58,59]

In summary, this case is a unique variation in the insertion of the SA, GL, ST and SM tendons with a very long tendon of the SA and ST. Surgeons should be aware of the present findings during the tendon harvesting procedure.

Conclusions:

The results of the present study revealed essential differences between ACL and tendons used as grafts for ACL reconstruction in regard of fibril–interstitium ratio and fibroblast . In terms of clinical relevance all the mentioned tendons can be used as auto or allografts for ACL reconstruction. Both ST and GL tendons provide significantly more density of

collagen fibrils as well as density of fibroblasts in comparison with patellar tendons. These findings provide a potential advantage of the hamstrings group on better remodeling and regeneration of the tissue. Patellar ligament has highest tensile load and more crimps than ST and GL tendons but with poor fibroblasts and less density of fibrils leading to less remodeling and regeneration. ST tendon has the highest number of fibroblasts between the tendons studied, while GL has the highest density of collagen fibrils. The present study recommends the use of coupling the ST and GL tendons for good remodeling and regeneration. The present study reports a case of a previously undescribed anatomical variant of the pes anserinus that have implications for tendon harvesting, preparation at ACL reconstruction and have an impact on the outcome of surgery.

References

1. Zantop T, Petersen W, Sekiya JK, Musahl V, Fu FH. Anterior cruciate ligament anatomy and function relating to anatomical reconstruction. Knee Surg Sports Traumatol Arthrosc. 2006;14(10):982-92.
2. Janssen KW, Orchard JW, Driscoll TR, van Mechelen W. High incidence and costs for anterior cruciate ligament reconstructions performed in Australia from 2003–2004 to 2007–2008: time for an anterior cruciate ligament register by Scandinavian model?. Scand J Med Sci Sports. 2012;22(4):495-501.
3. Standring S, editor. Gray's anatomy: the anatomical basis of clinical practice. 3. Edinburgh: Elsevier Churchill Livingstone; 2005: 1461.
4. Girgis FG, Marshall JL, Monajem A. The cruciate ligaments of the knee joint. Anatomical, functional and experimental analysis, Clin Orthop. 1975;106:216–31.
5. Odensten M and Gillquist J. Functional anatomy of the anterior cruciate ligament and a rationale for reconstruction, J Bone Joint Surg Am. 1985;67:257–62.
6. Amis AA and Dawkins GP. Functional anatomy of the anterior cruciate ligament. Fiber bundle actions related to ligament replacements and injuries. J Bone Joint Surg Br. 1991;73:260–67.
7. Indelli PF, Dillingham MF, Fanton GS. Anterior cruciate ligament reconstruction using cryo preserved allografts. Clin Orthop. 2004; 420:268–75.
8. Provenzano PP and Vanderby Jr R. Collagen fibril morphology and organization: implications for force transmission in ligament and tendon, Matrix Biology. 2006;25:71–84.
9. Hoffmann A and Gross G. Tendons and ligaments engineering in the adult organism: mesenchymal stem cells and gene-therapeutic approaches, International Orthopaedics. 2007;31:791–97.
10. Haraldsson BT, Aagaard P, Krogsgaard M, Alkjaer T, Kjaer M, Magnusson SP. Region specific mechanical properties of the human patella tendon, Journal of Applied Physiology. 2005;98(3):1006–12.
11. Benjamin M, Kaiser E, Milz S. Structure-function relationships in tendons: a review, Journal of Anatomy. 2008;212:211–28.
12. Hadjicostas PT, Soucacos PN, Koleganova N, Krohmer G, Berger I. Comparative and morphological analysis of commonly used autografts for anterior cruciate ligament reconstruction with the native ACL: an electron, microscopic and morphologic study. Knee Surg Sports Traumatol Arthrosc. 2008;16(12):1099-107.
13. Wren TA, Yerby SA, Beaupre GS, Carter DR. Mechanical properties of the human Achilles tendon, Clinical Biomechanics (Bristol, Avon). 2001;16:245–51.
14. Abramowitch SD, Woo SL, Clineff TD, Debski RE. An evaluation of the quasi-linear viscoelastic properties of the healing medial collateral ligament in a goat model, Annals of Biomedical Engineering. 2004; 32:329–35.
15. Beasley L, Weiland D, Vidal A, Chhabra A, Herzka A, Feng M, West R. Anterior cruciate ligament reconstruction: A literature review of the anatomy, biomechanics, surgical considerations, and clinical outcomes. Operative Techniques in Orthopaedics. 2005; 15(1): 5-19.
16. Mochizuki T, Akita K, Muneta T, Sato T. Pes anserinus: layered supportive structure on the medial side of the knee. Clin Anat. 2004;17(1):50-4.
17. Gu B, Fan Q, Lu Y, Goldenberg B. Repair of a huge defect of the gluteal region by rotation of a combined tensor fasciae latae sartorius myocutaneous flap. Plast Reconstr Surg. 1990;86:983-6.
18. Koshima I, Soeda S, Ohno A. Combined tensor fasciae latae musculocutaneous flap and sartorius musculocutaneous flap for the repair of wide defects of the lower leg. Plast Reconstr Surg. 1990;86:1198-201.
19. Kijkunasathian C, Limitlaohaphan C, Saengpetch N, Saitongdee P, Woratanarat P. The location of pes anserinus insertion in Thai people. J Med Assoc Thai. 2009;92 (6):S189-92.
20. Koulouris G and Connell D. Hamstring muscle complex: an imaging review. Radiographics. 2005;25(3):571-86.
21. Aherne WA and Dunhill MS. Point counting and the estimation of volume fraction. ***In***: Aherne WA, Dunhill MS, eds. Morphometry. 3rd Edn. London, Edward Arnold Publishers, 1987: 33–44.
22. Howard CV and Reed MG. Estimation of component volume and volume fraction. ***In***: Howard CV, Reed MG, eds. Unbiased Stereology. Three-Dimensional Measurement in Microscopy. 1st Edn. Oxford, BIOS Scientific Publishers, 1998: 55–65.
23. Järvinen TAH, Jozsa L, Kannus P, Järvinen TLN, Järvinen M. Organization and distribution of intramuscular connective tissue in normal and immobilized muscles. J Muscle Res Cell Mot. 2002; 23: 245-254.
24. Noyes F, Butler D, Grood E, Zernicke R, Hefti M. Biomechanical analysis of human ligament grafts used in knee-ligament repairs and reconstructions. Journal of Bone and Joint Surgery. 1984;66A:344-52.
25. Harner CD, Baek GH, Vogrin TM, Carlin GJ, Kashiwaguchi S, Woo SL. Quantitative analysis of human cruciate ligament insertions. Arthroscopy. 1999;15:741-49.
26. Jordan SS, DeFrate LE, Nha KW, Papannagari R, Gill TJ, Li G. The in-vivo kinematics of the anteromedial and posterolateral bundles of the anterior cruciate ligament during weight bearing knee flexion. Am J Sports Med. 2007;35:547-54.
27. Komzák M, Hart R, Okál F, Safi A. Does the posterolateral bundle influence rotational movement more than the anteromedial bundle in anterior cruciate ligament reconstruction?: A clinical study. J Bone Joint Surg Br. 2012;94(10):1372-6.

28. Takahashi M, Doi M, Abe M, Suzuki D, Nagano A. Anatomical study of the femoral and tibial insertions of the anteromedial and posterolateral bundles of human anterior cruciate ligament. Am J Sports Med. 2006;34:787–92.
29. Smith BA, Livesay GA, Woo SL. Biology and biomechanics of the anterior cruciate ligament. Clin Sports Med. 1993;12:637–70.
30. Hollis JM, Takai S, Adams DJ. The effects of knee motion and external loading on the length of the anterior cruciate ligament (ACL): A kinematic study. J Biomech Eng. 1991; 3:208-14.
31. Steckel H, Starman JS, Baums MH, Klinger HM, Schultz W, Fu FH. Anatomy of the anterior cruciate ligament double bundle structure: a macroscopic evaluation. Scand J Med Sci Sports. 2007; 17(4):387-92.
32. Takai S, Woo SL, Livesay GA: Determination of the in-situ loads on the human anterior cruciate ligament. J Orthop Res. 1993;11:686-95.
33. Franchi M, Quaranta M, De Pasquale V, Macciocca M, Orsini E, Trirè A, Ottani V, Ruggeri A. Tendon crimps and peritendinous tissues responding to tensional forces. Eur J Histochem. 2007;51(1):9-14.
34. Järvinen TA, Järvinen TL, Kannus P, Józsa L, Järvinen M. Collagen fibers of the spontaneously ruptured human tendons display decreased thickness and crimp angle. J Orthop Res. 2004;22(6):1303-9.
35. Szczesny SE, Peloquin JM, Cortes DH, Kadlowec JA, Soslowsky LJ, Elliott DM. Biaxial tensile testing and constitutive modeling of human supraspinatus tendon. J Biomech Eng. 2012; 134(2): 021004.
36. De Campos Vidal B. Image analysis of tendon helical superstructure using interference and polarized light microscopy, Micron. 2003; 34:423–32.
37. Raspanti M, Manelli A, Franchi M, Ruggeri A. The 3D structure of crimps in the rat Achilles tendon. Matrix Biol. 2005;24(7):503-7.
38. Bruehlmann SB, Rattner JB, Matyas JR, Duncan NA. Regional variations in the cellular matrix of the annulus fibrosus of the intervertebral disc.J Anat. 2002; 201(2): 159-71.
39. Frank CB. Ligament structure, physiology and function. J Musculoskelet Neuronal Interact. 2004; 4(2):199-201.
40. Cooper JA, Lu HH, Ko FK, Freeman JW, Laurencin CT. Fiber-based tissue-engineering scaffold for ligament replacement: design considerations and in vitro evaluation. Biomaterials. 2005; 26:1523–32.
41. Weiss M, Unterhauser FN, Weiler A. Crimp frequency is strongly correlated to myofibroblast density in the human anterior cruciate ligament and its autologous tendon grafts. Knee Surgery, Sports Traumatology, Arthroscopy. 2012; 20 (5): 889-95.
42. Petersen W and Tillmann B. Anatomy and function of the anterior cruciate ligament. Orthopade. 2002;31:710-8.
43. McBride DJ, Trelstad RL, Silver FH. Structural and mechanical assessment of developing chick tendon. International Journal of Biological Macromolecules. 1988;10: 194–200.
44. Silver FH, Christiansen DL, Snowhill PB, Chen Y, Landis WJ. The role of mineral in the viscoelasticity of turkey tendons. Biomacromolecules. 2000;1: 180–5.
45. Erisken C, Zhang X, Moffat KL, Levine WN, Lu HH. Scaffold fiber diameter regulates human tendon fibroblast growth and differentiation. Tissue Eng Part A. 2012. Abstract.
46. Rumian AP, Wallace AL, Birch HL. Tendons and ligaments are anatomically distinct but overlap in molecular and morphological features—a comparative study in an ovine model, Journal of Orthopaedic Research. 2007;25:458–64.
47. Chandrashekar N, Mansouri H, Slanterbeck J, Hashemi J. Sex-based differences in the tensile properties of human anterior cruciate ligament. J Biomech. 2006; 39:2943–50.
48. Yin L and Elliott DM. A biphasic and transversely isotropic mechanical model for tendon: application to mouse tail fascicles in uniaxial tension. J Biomech. 2004;37(6):907-16.
49. Butler DL, Kay MD, Stouffer DC. Comparison of material properties in fascicle-bone units from human patellar tendon and knee ligaments. J Biomech. 1986;19(6):425-32.
50. Woo SL, Wu C, Dede O, Vercillo F, Noorani S. Biomechanics and anterior cruciate ligament reconstruction. J Orthop Surg Res. 2006;1: 2.
51. Mologne TS and Friedman MJ. Graft options for ACL reconstruction. American Journal of Orthopedics. 2000; 29: 845-53.
52. Brown CH and Sklar JH. Graft selection: Nonpatellar alternatives gain popularity. Biomechanics. 1998:21-25.
53. Barrett, GR, Boojin FK, Hartzog CW, Nash CR. Reconstruction of the anterior cruciate ligament in females: A comparison of hamstring versus patellar tendon autograft. Arthroscopy: Journal of Arthroscopic and Related Surgery. 2002;18: 46-54.
54. Ivey M and Prud'homme J. Anatomic variations of the pes anserinus: a cadaver study. Orthopedics. 1993;16:601–6.
55. Beltran J, Matityahu A, Hwang K, Jbara M, Maimon R, Padron M, Mota J, Beltran L, Sundaram M. The distal semimembranosus complex: normal MR anatomy, variants, biomechanics and pathology. Skeletal Radiol. 2003;32(8):435-45.
56. Stoane J M and Gordon D H. MRI of an accessory semimembranosus muscle. J Comput Assist Tomogr.1995;19(1):161–2.
57. Cattrysse E, Barbaix E, Janssens V, Alewaeters K, Van Roy P, Clarijs JP. Observation of a supernumerary hamstring muscle: a state of the art on its incidence and clinical relevance.Morphologie. 2002; 86(274):17-21.
58. Tang ML, Liu XY, Ren JW, Zhang DC, Li RS, Wen YM, Ge BF. The sartorius myocutaneous island flap. Surg Radiol Anat. 1993;15(4):259-63.
59. Clavert P, Cognet JM, Baley S, Stussi D, Prevost P, Babin SR, Simon P, Kahn JL. Anatomical basis for distal sartorius muscle flap for reconstructive surgery below the knee. Anatomical study and case report. J Plast Reconstr Aesthet Surg. 2008;61(1):50-4.

12/26/2012