Comparative and Morphological Analysis of Patellar Tendon with the Native Anterior Cruciate Ligament (ACL): an Electron, Microscopic and Morphological Study

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Background: Ligaments and tendons are similar in composition but differ in proportion and arrangement. Tendons are used as grafts for the ACL reconstruction. The microscopic structure of these tendons has not been sufficiently studied and compared to the native ACL.
Objective: To compare the structure of the patellar tendon graft with the structure of a normal anterior cruciate ligament.
Material and methods: A null hypothesis was declared stating that the anterior cruciate ligament should be histologically, morphologically and functionally different from the patellar tendon used for ACL reconstruction. We investigated similarities and differences of the structure of ACL and patellar tendon used as a graft tissue for ACL reconstruction. In this study, samples of patellar tendon, and the ACL were harvested from 18 patients during ACL reconstruction and analysed by light and electron microscopy, immunohistochemistry and morphometry.
Results: The thickness of the collagen fibrils, collagen organization and diameter, the fibril/interstitium ratio, density of fibroblasts and blood vessels, and distribution of the collagen type I, III and V fibrils were analyzed.
Discussions: The ACL had the highest concentration of type III and V collagen fibrils as well as elastic fibers.
Conclusion: The histological and ultrastructural appearance of the ACL differs from patellar tendon used as graft for ACL reconstruction.

Keywords: ACL reconstruction, patellar tendon, comparative morphology, morphometry, immunohistochemistry

Introduction

The anterior cruciate ligament is responsable for the anterior translation of the tibia relative to the femur [1,2]. The primary goal of anterior cruciate ligament (ACL) reconstruction is to restore normal knee function, eliminate the instability and minimize degenerative joint changes [3]. In case of the ACL reconstruction, two types of graft tendons are mostly used. These are the autologous bone-patellar tendon-bone graft, the semitendinosus with or without the gracilis.

In this study, we examined the histological and morphological features of the ACL and the graft tissues of patellar tendon that are used in reconstruction of the anterior cruciate ligament. The aim was to compare the patellar tendon graft with the structure of a normal anterior cruciate ligament. We hypothesized that the patellar tendon grafts are significantly different in terms of histological and morphological composition to the ACL.

Previous studies have compared the different tendon grafts on the basis of their biomechanical strength, fixation method, surgical technique and structure differences [4,5,6]. In our study we used fresh tendons of patients who underwent an ACL reconstruction. Therefore the results of this study are not influenced by potential changes due to age, lack of use, injury or possible artifacts related to freezing of specimens.

Material and method

Experimental set-up

Eighteen human patellar tendons and the anterior cruciate ligament were used. Specimens were obtained from patients who underwent ACL reconstruction surgery. There were 12 male and 6 female patients, with a mean age of 28.6 years (range 18–41 years). The ACL tissues were removed arthroscopicaly and the patellar tendon in an open manner to prevent damaging the tendons during the harvesting process. The central third of patellar tendon and quadriceps tendons at a distance of 1 cm proximal and distal from the patellar bone were removed from one knee of the 18 patients. For the ACL, the central portion was used in this study. To minimize dehydration, each specimen was immediately wrapped in saline-moistened paper towels, followed by plastic wrap in aluminium foil, and then stored at –30 °C.

Histology

All tissues were investigated by light and electron microscopy, immunohistochemistry and were additionally analyzed by morphometry. For light microscopy the removed specimens were formaldehyde fixed (4% formaldehyde in phosphate-buffered saline), paraffin-embedded via routine procedure. The size of all the studied probes was 20 mm ×

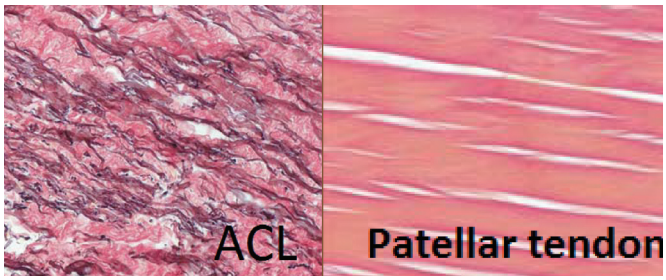

Fig. 1. Expression of collagen III. Immunohistologic staining for collagen III

10 mm × whole thickness of tendons for the patellar tendon and 20 mm × whole for the ACL.

Transmission electron microscopy

Tendons were placed in 4% formaldehyde, and then sent to cut and embedded for transmission electron microscopy. The cross sections (100 nm thick) of each fixed tendons were rinsed in 0.1 M phospahate buffer then placed in 1% osmium tetroxide in 0.1 M phosphate buffer for 2 h. They were then dehydrated in graded ethanol solutions and transferred to propylene oxide. The infiltration process was performed for 24 h, and then the specimens were hardened at 40 °C for 48 h. Thin sections, three from the center of each tendon studied, were stained with aqueous uranyl ace-tate and lead citrate, and then scanned. Ten random fields were photographed from each section at a magnification of 10,000 ×. The samples of patellar tendon and the ACL were collected, and processed for (1) measurement of collagen fibril diameter, (2) fibril density (fibril-interstitium ratio), (3) density of blood vessels (4) density of fibroblasts, (5) percentage of elastic fibrils and (6) percentage of the collagen type I, III and V. All specimens were investigated by immunohistochemistry. The CD34 marker, which stains in the endothelial cells, was used to identify blood vessels within the tendinous tissue. The CD34 antibody (IgG1; human anti mouse antibody; dilution 1:25) was used for this type of staining. Primary antibodies were diluted in commercially available antibody Diluent (Dako ChemMate-TM) and detection was carried out using the Dako ChemMate-TM kit. Non-binding monoclonal mouse IgG1 was used as a negative control. The sections were finally counterstained with hematoxylin and mounted. For immunohistochemical stainings for collagen the following antibodies were used: collagen type I (rabbit polyclonal), collagen type III (rabbit polyclonal), collagen type V (rabbit polyclonal 1:300). Briefly, paraffin sections were deparaffinated in xylol and rehydrated through grading alcohol concentrations and incubated with primary antibodies for 60 min. After rinsing in PBS, sections were incubated with secondary antibodies for 30 min and finally with streptavidin-conjugated alkaline phosphatase (Biogenex, SanRamon, USA) for 30 min. All steps were performed at room temperature. Negative controls were performed by omitting the primary antibody. For detection of elastic fibrils, Elastica Van Gieson (EVG) staining was used. The following parameters were analyzed by morphometry: fibrils–interstitium ratio (%); thickness of fibrils (nm), density of blood vessels (number of blood vessels per 1 mm^2 of tendinous tissue), density of fibroblasts (number of fibroblasts per 1 mm^2 of tendinous tissue), percentage in tendinous tissue of collagen and elastic fibrils. The measurement was performed on the whole length of the section. The density of blood vessels was defined as the number of blood vessels per 1 mm^2 of tendinous tissue. This measurement was performed on sections stained with CD34. Positive stained vessels were counted in the whole area section. The average number of blood vessels per 1 mm^2 of tendinous tissue was calculated. The density of fibroblasts was determined on H&E stained sections. The number of fibroblasts was counted in the whole area section. Finally, the number of fibroblasts per 1 mm^2 of tendinous tissue was calculated. The percentage of elastic fibrils was analyzed in EvG staining (Fig. 1). The percentage of collagen fibrils was analyzed in each immunohistochemical staining. Statistical evaluation Data are given as mean±SD. For the statistical analisys SPSS softwear was used. The results were considered significant when the probability of error (p) was lower than 0.05.

Results

The microstructure of the ACL is similar to the patellar tendon graft although distinct differences exist in the his-

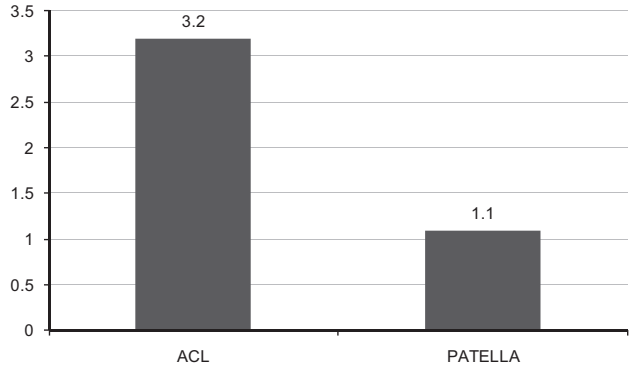

Fig. 2. Density of blood vessels (/mm^2)

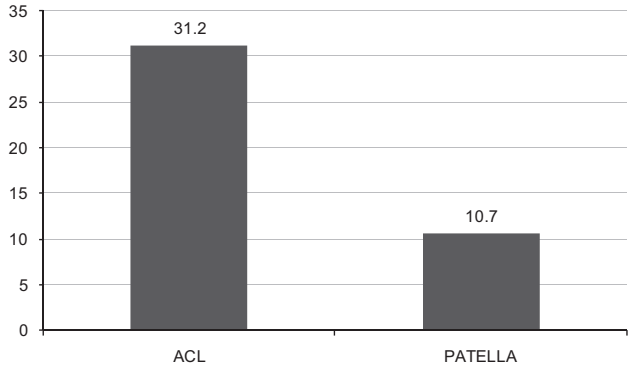

Fig. 3. Density of fibroblasts (/mm^2)

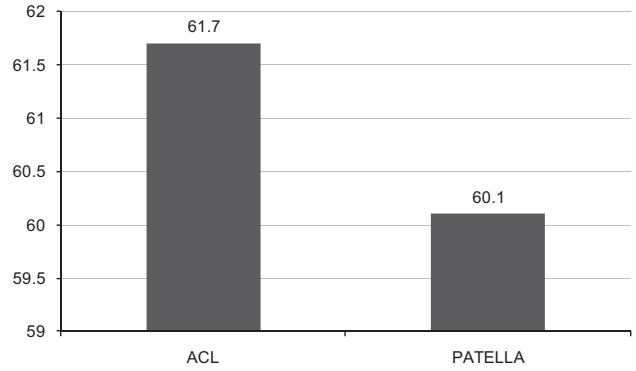

Fig. 4. Fibril-interstitium ratio

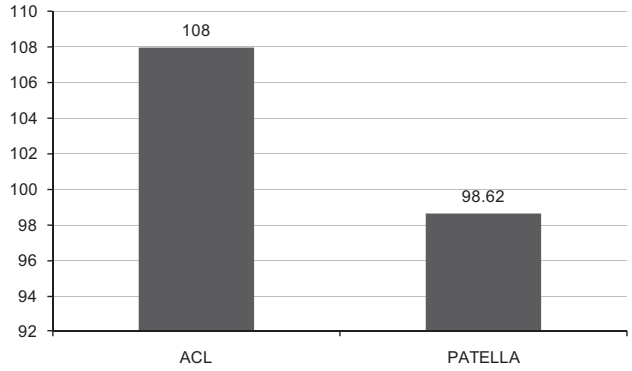

Fig. 5. Thickness of the collagen fibrils

tologic and electron microscopic preparations. Light and scanning electron microscopy revealed a combination of a helical and planar wave pattern for ACL fibrils. Thus, there is a combination of parallel or twisted, nonlinear networks. The cell bodies of the fibroblasts in the ACL appear elongated. Microscopically, all the patellar tendons studied are composed of closely packed collagen bundles in intracellular matrix of proteoglycan. Fibroblasts were the predominant cell type and are arranged in parallel rows between bundles of parallel arranged collagen fibrils. The cell bodies of the fibroblasts in the ACL appear elongated. The density of blood vessels per 1 mm^2 was 1.1 for the patella tendon. On the other hand, the ACL showed the richest vascularity having 3.2 blood vessels per 1 mm^2 (Fig. 2). The density of fibroblasts per 1 mm^2 of collagen fibrils was for the patellar tendon 10.7±1.7. The ACL showed the highest concentration of fibroblasts, being 31.2±9.3 (Fig. 3). The analysis of the fibril–interstitium ratio was in the patella tendon 60.1. The ACL showed a 61.7% (Fig. 4). The thickness of the collagen fibrils was approximately equal in all the tendon grafts and the ACL. Patella had an average thickness of 98.62±57.42. The ACL collagen thickness was 108±43.1. The anterior cruciate ligament showed the highest density of elastic fibers in the tissues studied, being 3.35% (Fig. 5). The elastic fibers in the patella was 0.17%±0.13. The patella showed a density of collagen I fibers of 71.7%, and the ACL 27.5%. The patella, showed a density of collagen type III fibers of 16.1%, while in the. ACL showed the highest density, being 49.8%. The ACL also showed the highest density of collagen V fibers (29.1%) among the patellar tendon (13.4%) (Fig. 7).

Discussion

The anterior cruciate ligament has a unique and complex histological and ultrastructural structure, which cannot be replaced by any kind of the tendon grafts. A second qualification of our data relates to the fact that this is only a histological, electron and morphological study, aiming to find any similarities and differences in these tissues, which may be important as tissue grafts. Our cross sectional measurements area of 1 mm^2 of tissue studied, the highest density of fibroblasts was found in the anterior cruciate ligament. Cooper et al. [7] showed that the tendon fibroblasts proliferated faster than the ACL fibroblasts regardless of the material and geometry. Pufe et al. [8] noted that the fibroblasts density influences the mechanical properties of the tendon negatively. Tohyama et al. [9] showed inferior mechanical properties of the residual and the regenerated tissues for up to 24 weeks after removal of the central portion in the patellar tendon. During tissue repair, growth factors stimulate fibroblasts proliferation and synthesis of type I and III collagen fibrils. In the early phases of ligament healing and remodeling, more type III collagen is produced than type I. The anterior cruciate ligament, showed the smallest percentage of collagen type I, but the highest type III and V among the patellar

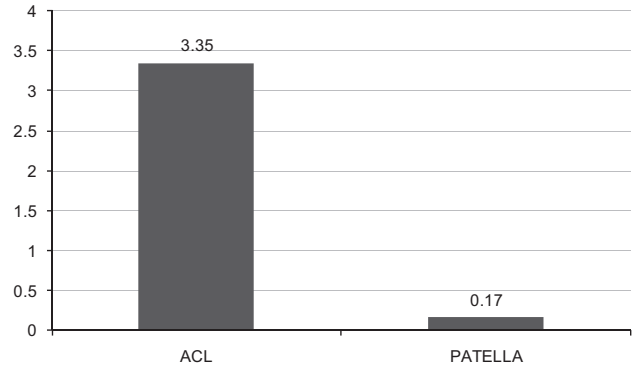

Fig. 6. Density of elastic fibers (/mm^2)

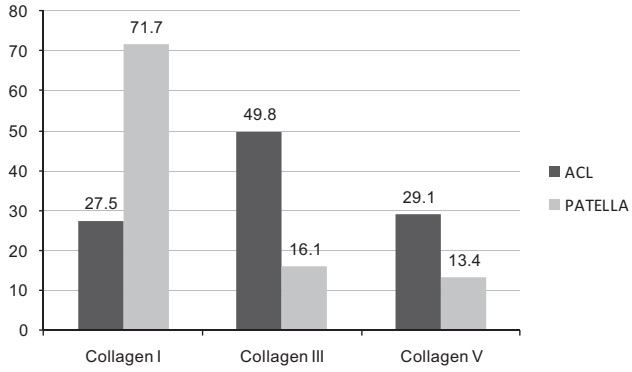

Fig. 3. Density of collagen I, III, V

tendon. Within the ACL tissue, the presence of collagen type I collagen is closely related to tensile strength [10]. The ACL also showed the highest concentration of elastic fibrils. This allows it to withstand multiaxial stresses and varying tensile strains. The elastic fibrils system may affect the tendon strength. Abnormalities in elastic fibrils, and specifically in the fibrillin component, have been noted in Marfan's syndrome and adolescent idiopathic scoliosis, conditions that are associated with connective tissue laxity [11,12,13]. The results of our study also showed that the anterior cruciate ligament had the highest concentration of blood vessels among the patellar tendon. Biomechanical evaluation of free tendon grafts showed correlation of graft strength and vascularization [14]. Parry et al. [15,16] have showed that collagen fibrils in tendon and ligament usually have a bi- or trimodal distribution in term of diameter, and that the distribution correlated with structure's mechanical properties. Other investigations have shown that collagen fibril diameter distribution alone cannot predict the material and structural properties of a tendon [17,18,19]. Our study also showed that there was an absence of any significant difference in the collagen fibril diameter, both between the patellar tendon and the ACL. Several studies have correlated a decrease in the mean collagen fibril diameter with a loss of mechanical properties in healing and remodeling of ligaments and tendons [20,21]. The patellar tendon did not showed a higher fibril–interstitium ratio in comparison with ACL. A high percentage of the collagen fibrils in tendons can attribute essentially to strength of this tissue and can be important for the biomechanical capacity of tendinous grafts [22]. On the other hand, a high quote of the collagen fibrils can influence negatively the elasticity and constriction of tendons, an important functional factor as ACL-grafts [23,24].

Conclusion

The results of our study revealed essential differences between ACL and patellar tendon used as grafts for ACL reconstruction in regard of fibril-interstitium ratio, fibroblast and blood vessels density as well as a content of elastic fibrils. ACL showed a highest percentage of the elastic fibrils, fibroblasts and blood vessels and a relatively low fibrils/ interstitium ratio, parameters which are important for the ACL function as ligament.

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