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Histological analysis of cross-sectional area of quadruple hamstring tendons and patellar ligament samples in relation to age and gender

Egon Biuk [a,e,*], Zoran Zelić [a,e], Saša Rapan [a,e], Ivan Lovric [b,f], Dubravka Biuk [c,g], Radivoje Radic [d]

[a] Department of Orthopaedics, Osijek University Hospital, Josipa Huttlera 4, HR-31000 Osijek, Croatia
[b] Clinic for Surgery, Osijek University Hospital, Josipa Huttlera 4, HR-31000 Osijek, Croatia
[c] Department of Ophthalmology, Osijek University Hospital, Josipa Huttlera 4, HR-31000 Osijek, Croatia
[d] Department of Anatomy and Neuroscience, Faculty of Medicine, JJ Strossmayer University of Osijek, Cara Hadrijana 10 E, HR-31000 Osijek, Croatia
[e] Department of Orthopaedics, Faculty of Medicine, JJ Strossmayer University of Osijek, Cara Hadrijana 10 E, HR-31000 Osijek, Croatia
[f] Department of Surgery, Faculty of Medicine, JJ Strossmayer University of Osijek, Cara Hadrijana 10 E, HR-31000 Osijek, Croatia
[g] Department of Ophtalmology, Faculty of Medicine, JJ Strossmayer University of Osijek, Cara Hadrijana 10 E, HR-31000 Osijek, Croatia

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ABSTRACT

Aims: The middle of the patellar ligament and the quadruple hamstring tendons (gracilis and semitendinosus) are two types of graft predominantly used in anterior cruciate ligament (ACL) reconstruction. The aim of this study was to determine the morphometric characteristics of patellar ligament grafts and hamstring tendon grafts and to compare the results according to subject age and gender.
Materials and methods: The study was conducted on a total of 120 samples: 40 of gracilis tendon, 40 of semitendinosus tendon and 40 of patellar ligament, distributed equally according to gender, age (50–75 years) and the side of the body from which the sample was harvested.
Results: Morphometric and histological analyses showed that patellar ligament samples had less cross-sectional area than quadruple tendon samples (49.29 mm^2 compared with 51.46 mm^2, respectively). Sexual dimorphism was noticed in distal cross-sections of gracilis tendons ($p = 0.09$), cross-sections of quadruple tendons ($p = 0.07$) and patellar ligament samples ($p = 0.01$) because of different muscular build.
Conclusions: All samples obtained from male subjects had larger cross-sectional areas compared with the samples taken from females. Furthermore, samples obtained from subjects aged 60 years or under had larger cross-sectional areas than samples obtained from subjects aged at least 61 years for all types of graft.

Introduction

Tendons are pearly white-coloured connective tissue structures in the form of a sheet or band. Muscular contraction forces are transferred through tendons to a bone or another structure to which a muscle is attached and acts on. Tendons have different forms and dimensions depending on the type, size and strength of the associated muscles. The location and distance of the point of insertion of the tendon relative to the joint axis determine the lever arm of the moment of force that is being exerted on a joint. An example is the quadriceps muscle tendon, which acts on the knee joint through the patella and patellar tendon [1,2].

Ligaments connect the ends of bones that form joints and help to maintain joint stability. They control the relative motion of the bones in the joint and, unlike muscles and tendons, provide passive stability to the joint. Not all ligaments become equally taut at different stages of flexion and extension: for example, cruciate ligaments in the knee joint have interwoven and overlapping fibres so that some ligaments are always taut, while the others are lax, thus ensuring constant stable contact between different parts of the knee joint [3,4].

Tendons, ligaments, aponeuroses and other similar connective tissue structures are composed of dense bundles of collagenous fibres ordered in parallel, slightly wavy or curved arrays. About 20% of the mass of both tendons and ligaments is made up of cellular component, mostly fibrocytes and fibroblasts, and about 80% is extracellular component [5,6]. Between 60% and 80% of their total mass is made up of water; the remaining 20% to 40% is solid material. Collagen fibres make up between 65% and 80% of the dry

* Corresponding author at: Department of Orthopaedics, Faculty of Medicine, JJ Strossmayer University of Osijek, Cara Hadrijana 10 E, HR-31000 Osijek, Croatia.
E-mail address: egon.biuk@gmail.com (E. Biuk).

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mass of tendons and ligaments and are mostly type 1 collagen with much less type 3 collagen [7]; however, collagen content is higher in tendons than in ligaments. There is also a difference in the ratio of collagen type 1 to type 3, which varies between 95%: 5% and 99%: 1% in tendons, and is 90%: 10% in ligaments. Both tendons and ligaments in the extremities contain little elastin (around 3%) [8–10].

Tendons and ligaments are used in reconstructive surgery. For example, in reconstruction of anterior cruciate ligament (ACL) in the knee joint, the ACL is commonly replaced by two potential autografts: patellar ligament graft and quadruple hamstring tendon graft. Therefore, it is imperative to study the structure of ligaments and tendons to determine their properties and shape; this information will help surgeons to choose the optimal graft.

The aim of this study was to determine the morphometric characteristics of patellar ligament grafts and quadruple hamstring tendon grafts.

Materials *and methods*

This study included 40 samples each of patellar ligament, semitendinosus tendon and gracilis tendon obtained from the Department of Anatomy and Neuroscience collection of anatomical preparations at the Faculty of Medicine, J.J. Strossmayer University of Osijek. All samples were obtained from individuals aged between 50 and 75 years.

Patellar ligament graft was harvested from the middle part of the patella ligament and was 10 mm wide, with a 10 mm wide and 25 mm long patellar bone plug and a 10 mm wide and 30 mm long tibial bone plug [11–13] (Fig. 1). Bone plugs were formed by passing through a cylinder of 10 mm in diameter and fixing both ends with sutures through holes in the bone [14–17] (Fig. 2). A tissue sample for histological analysis was taken from the central part of each patellar ligament graft.

Quadruple hamstring tendon graft was obtained from folded semitendinosus and gracilis tendons. After their biomechanical characteristics were determined, a sample was taken for histological analysis from the proximal, middle and distal part of each hamstring tendon graft, as was done for the patellar ligament graft. Cross-sectional areas were calculated as arithmetic mean of area values of proximal, central and distal cross-sections of tendons.

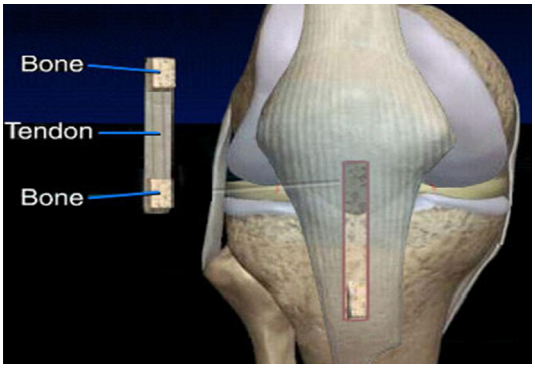

Fig. 1. Patellar ligament graft.

Fig. 2. Patellar ligament graft with bone plugs.

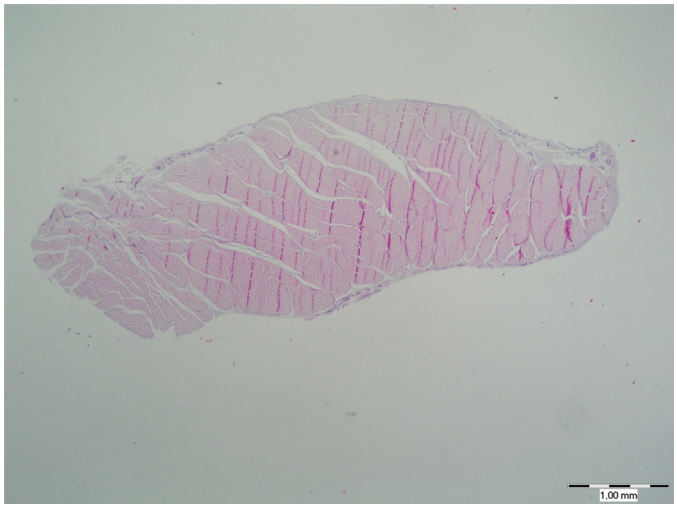

Fig. 3. A sample prepared for histological analysis.

Histological analysis was conducted to determine cross-sectional areas of the samples and the amount of force per unit area of dense connective tissue. Formalin-fixed, paraffin-embedded tissue samples were cut in a microtome to 6-μm-thick preparations and then stained using haematoxylin-eosin technique [6] (Fig. 3).

Histological samples were photographed using a digital camera Olympus C-5050 mounted on an Olympus BX50 microscope. Quick Photo Pro software was used. A special type of Ellipse software, PointGrid, produced by ViDiTo, was used to measure cross-sectional area. This programme uses a virtual point grid on a calibrated photo: multiplying the number of points projected onto the sample by the area allotted to each point gives the area of the sample (Fig. 4.).

Results

Morphometric and histological analyses showed that patellar ligament samples had less cross-sectional area than quadruple tendon samples (49.29 mm^2 and 51.46 mm^2, respectively; $p = 0.01$); the differences between cross-sectional areas of semitendinosus and gracilis tendons were highly statistically significant ($p < 0.001$) (Table 1.).

Analysis of sexual dimorphism showed that there were statistically significant differences in patellar ligament samples: male samples had larger cross-sectional areas than female samples ($p = 0.01$) (Table 2.).

All hamstring tendon samples and the patellar ligament samples obtained from subjects aged 60 years or under had larger cross-sectional areas than samples obtained from subjects aged at least 61 years (Table 3.).

Samples taken from the left side of the body had slightly greater values than samples taken from the right side (Table 4.).

Discussion

Measurement of sample thickness is important for assessing the biomechanical properties of samples and is a potential weak spot in this type of research that can cause considerable error [18,19]. Taking this into account, we tried to measure cross-sectional areas of samples as precisely as possible, and in samples of semitendinosus and gracilis tendons the measurements were taken at three levels to diminish the influence of potential difference in proximal and distal ends of tendons. The use of a special edition of the Ellipse software, PointGrid, minimised the probability of error in this phase of the research.