

Light microscopic histology of quadriceps tendon ruptures

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Abstract

Purpose To assess histological changes and possible differences in the quadriceps of patients undergoing open repair of the tendon after spontaneous rupture, and subjects with no history of tendon pathology.

Materials Biopsies were harvested from the quadriceps tendon of 46 patients (34 men, 12 women) who had reported unilateral atraumatic quadriceps tendon rupture and had undergone surgical repair of the tendon. Samples were also harvested from both the tendons in 11 ($N=11\times 2$) patients, nine males and two females, dying from cardiovascular disorders. For each tendon, three slides were randomly selected and examined under light microscopy, and assessed using a semiquantitative grading scale (range 0–21) which considers fibre structure, fibre arrangement, rounding of the nuclei, regional variations in cellularity, increased vascularity, decreased collagen stainability, and hyalinisation.

Results The pathological sum-score averaged 19.2 ± 3.7 in ruptured tendons and 5.6 ± 2.0 in controls, and all variables considered were significantly different between the two groups, showing an association between tendon abnormalities and rupture ($0.05 < P < 0.001$).

Conclusion This study confirms that the presence of histological degenerative changes in torn quadriceps tendons increases the risk of rupture.

Introduction

Relatively frequent in older patients with systemic disease, quadriceps tears are the second most common injury to the extensor mechanism of the knee after patellar fractures [1]. The mechanism of injury is usually a forced contraction of the quadriceps with the knee flexed and the foot fixed to the ground, but the pathogenesis is considered multifactorial, and is associated with disorders such as renal insufficiency [2], primary or secondary hyperparathyroidism, and other conditions which impair and weaken the osteotendinous junction [3]. In addition, obesity [4], corticosteroids injections [5], anabolic steroids [6] and statins [7] may also be predisposing factors, especially in atraumatic or minimally traumatic ruptures [8]. Genetic profiling has identified that the BstUI polymorphism of the COL5A1 is associated with bilateral ruptures of the quadriceps tendon [9].

In normal conditions, the tendon is resistant to tensile strain forces [10], and spontaneous ruptures occur in abnormal tendons, with evidence at histology of narrowing and obliteration of small arteries, hypertrophy of the intima and media walls [11].

We assessed at histology biopsies of quadriceps tendons harvested from two groups of patients: patients undergoing open repair of the tendon after spontaneous rupture, and patients with no history of tendon pathology.

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Materials and methods

All procedures described in this study were approved by our local Ethics Committee.

Tendon samples

Biopsies were harvested from the quadriceps tendon of 46 patients (34 men, 12 women; mean age, 51.3 ± 22 years, age

range 28–81 years) with no history of metabolic or systemic disorders who had reported unilateral atraumatic quadriceps tendon rupture and had undergone surgical repair of the tendon between 1996 and 2008. During surgery, always performed within 48 hours from injury, two samples approximately of 3×3×3 mm were harvested from the proximal and distal stumps of the tendon, immediately adjacent to the edge of the torn tendon.

We harvested samples from both the tendons in 11 ($N=11\times 2$) patients, nine males and two females (mean age, 74.5 ± 11.5 years; age range 66–91) who had died from cardiovascular disorders. The whole quadriceps tendons were harvested in the *post mortem* room, under sterile conditions, through a midline approach. After removal of surrounding tissues, we cut the tendons horizontally, at the superior and inferior ends. From available notes, none of these patients had experienced acute or chronic injuries to the quadriceps tendon, nor had taken corticosteroid drugs or fluoroquinolones in the last two years before death.

Staining procedures

All the samples were placed in 20 mL of sterile 10 % formalin in a universal container. In the deceased patients, a 3×3×3 mm sample was harvested 2.5 cm proximally to the patellar insertion of the tendon, and then fixed in 10 % neutral-buffered formalin (for 24–48 h), and processed in paraffin wax. Transverse 5- μ m sections were mounted onto 3-aminopropyltriethoxysilane-coated slides, and dried at 37 °C overnight. The sections were dewaxed in two ten minute changes of xylene, one change in absolute alcohol, one in 95 % alcohol and one in 70 % alcohol, for ten minutes each. Once sections had been rinsed under running tap water, they were stained with haematoxylin and eosin.

Assessment of tendon characteristics

For each tendon, three slides were randomly selected and examined under light microscope (3600, SM-LUX, Leitz, Wetzlar, Germany) by a senior clinician who was fully trained in the semi-quantitative histopathological assessment techniques used in this study. The identification number on each slide was covered with a removable sticker, and each slide was randomly numbered. Once one of the authors examined all the slides, the stickers were removed and a new sticker was applied, renumbering the slides using a new series of randomly generated numbers. The same author assessed again the degree of staining, and both findings were compared. In case of inconsistency (more than two grades on the scoring system described in Table 2) between the two findings, a consultant pathologist examined the slides. We selected for the

study the area with the most advanced status of disease, and used the more markedly abnormal pathological slides.

We point out that we harvested the whole of the right and left quadriceps tendons of the control groups, and we proceeded to stain the whole tendon. For the purposes of this study, we randomly selected for the semi-quantitative assessment described below samples from the control group taken from the area located at the same level from which the samples from the torn quadriceps tendons were taken.

To assess the slides, we used a semiquantitative grading scale ranging from 0 to 21 [12], which considers fibre structure, fibre arrangement, rounding of the nuclei, regional variations in cellularity, increased vascularity, decreased collagen stainability, and hyalinisation. Each variable is scored from 0 to 3 (0 is normal, 1 slightly abnormal, 2 abnormal, and 3 markedly abnormal) [13].

Statistics

The kappa statistic was used to assess the agreement between slide findings [14]. The chi-square test was used to ascertain the association between score and tendon type, control or ruptured. The Mann–Whitney *U*-test was used to compare the score difference between the two tendon groups. The SPSS (release 6.0) statistical package (SPSS, Chicago, Illinois) 36 was used. A p -value < 0.05 was considered to be statistically significant.

Results

The pathological sum-score averaged 19.2 ± 3.7 in ruptured tendons and 5.6 ± 2.0 in controls (Table 1). All variables considered were significantly different between the two groups, with an association between tendon abnormalities and rupture (Mann–Whitney *U*-test $0.05 < P < 0.001$) (Table 2). The kappa values, i.e. the agreement between the two readings, ranged from 0.61 to 0.88 (Table 3).

Table 1 Summary of pathological scores of control and ruptured quadriceps tendons

Total tendon pathologic score	Control tendons	Ruptured tendons
Mean	5.6	19.2
Median	4	18
SD	2.0	3.7
Range	0–16	12–21

The worst scoring result was used for each situation

Table 2 Distribution of tendon pathological scores

Variable	Control tendon (N=11×2)				Ruptured tendon (N=46×2)			
	0	1	2	3	0	1	2	3
Fibre structure	10	7	3	2	3	5	26	58
Fibre arrangement	9	6	4	3	4	6	22	60
Rounding of the nuclei	10	5	5	2	7	11	24	50
Regional variations in cellularity	13	4	4	2	5	8	28	51
Increased vascularity	12	6	2	1	2	13	25	52
Decreased collagen stainability	8	8	4	1	8	12	26	46
Hyalinisation	11	6	5	0	5	11	28	48

The worst scoring result was used in each situation

Variables

Fibre structure The control tendons showed parallel, closely packed collagen fibres and slight waviness of the fibre. Increased waviness and separated fibres were typical of mild moderate changes, where loss of the structure and marked hyalinisation were considered as marked abnormalities. The median score was 0.5 in control samples and 3 in ruptured tendons.

Fibre arrangement Fibres were parallel in control tendons, and differently disordered and arranged in ruptured biopsies, with a median score of 0.5 and 3 respectively.

Tenocyte nuclei Control tenocytes had flattened or spindle-shaped nuclei, arranged in rows, between the collagen fibres. Abnormal tenocytes had evidence of decreased or increased number of rounded nuclei. The median scores were 3 for ruptured tendons and 0 in controls.

Cellularity Ruptured tendons contained areas of increased cellularity compared to controls, showing a median score of 3, an index of marked cellularity. The median score in controls was 0.

Table 3 Kappa scores for each variable

Tendons	FS	FA	N	RVC	V	DCS	H
All	0.65	0.88	0.70	0.64	0.77	0.66	0.64
Ruptured	0.70	0.89	0.71	0.61	0.74	0.65	0.68
Control	0.62	0.84	0.68	0.69	0.80	0.69	0.62

1 indicates a perfect match and 0 represents no match

FS fibre structure, *FA* fibre arrangement, *N* rounding of the nuclei, *RVC* regional variations in cellularity, *V* increased vascularity, *DCS* decreased collagen stainability, *H* hyalinisation

Vascularity Normally, vascular bundles run parallel alongside collagen fibres. In ruptured tendons, the number of vessels was significantly greater than in controls, recording a median score of 3 and 0, respectively.

Collagen stain ability The normal collagen colour is pink-red when haematoxylin and eosin stains are added. In degenerative conditions, the stainability at histology was reduced, and the collagen looked paler (from 0 to 3). The median value was 1 for the control tendons, and 3 for ruptured. Grade 3 chondrocyte-like changes were evident in 11 control patients.

Discussion

We examined histologically samples of quadriceps tendons from two different populations of patients to assess the presence of histological changes, and ascertain whether tendon ruptures predispose to develop these changes or occur in already degenerated tendons. Interestingly, although our control group patients were on average 25 years younger than patients of the study group, mean and median scores were significantly lower in the control than in the study group. We found significant inter-group differences for each variable, with higher incidence of tendon changes in younger patients with tendon ruptures than in control individuals, even though these changes are often considered age related, and that older patients were more likely to develop them, probably because of impaired blood supply [15].

Jarvinen et al. [16] compared samples from spontaneously ruptured and apparently healthy tendons, and used the collagen crimp angle as a marker of tendon abnormality, showing a significantly greater concentration of degenerative changes after ruptures of the Achilles and biceps tendons, but they did not study the quadriceps tendons specifically. Another study [11] that analysed 29 spontaneous ruptures of the quadriceps tendon found that all ruptures contained hypoxic degenerative changes, which, indeed, were present in 34 % of the controls. In a retrospective series of 42 patients (45 quadriceps tendon ruptures), Trobisch et al. [17] reported that ruptures are more frequent between the sixth and seventh decade, and men are six times more predisposed than women to these injuries, concluding that the ratio degenerative–non-degenerative tendons is increasing with age, and quadriceps tendon tears may also occur in subjects without any degeneration to the tendon. In the study by Trobisch et al. [17], samples were harvested from the proximal stump of the rupture; we harvested two samples, from both the proximal and distal stumps, before repairing the lesion [18]. They used haematoxylin and eosin, van Gieson and Alcian blue stain, and evaluated the presence of degenerative changes such as organised scar tissue,

calcification, neovascularisation, chondrogenic metaplasia, severe mucoid collagen structures, and reduced cellularity, without using any validated histological scoring system. We stained the sections using haematoxylin and eosin, and applied a semiquantitative grading scale which considers different features of tendon degeneration, including structure and arrangement of fibres, nuclear aspect, increased or decreased cellularity, vascular changes, collagen stainability, and hyalinisation [19, 20]. Our study did not reveal any significant difference between samples harvested from the superior and inferior tendon stumps. A high score, expected in tendon degeneration, could be associated with aging, but we do not know whether these changes are expression of the normal aging process of the tendon, or increase the likelihood of incurring to tendon ruptures. The changes we observed could have occurred after rupture, but we operated on all the patients within 48 hours from injury, and we believe this time is relatively short for developing such changes. The reason why 75-year-old patients with no history of tendon disorders had significantly less tendon abnormalities than significantly younger patients (mean age of 51 years) who had reported tendon rupture could be that mechanical stresses may further damage and stretch tendons that are themselves abnormal and exceed their tensile strength, up to the point of rupture.

At histology, the intratendinous changes of the tendon stumps proximal and distal to the rupture were similar to those observed in Achilles tendon ruptures, and in chronic Achilles, patellar and rotator cuff tendinopathies [21, 22].

Even though our control patients had no evidence of histological degenerative changes, about one-third of the general population presents signs of tendon degeneration without any clinical evidence of discomfort [23]. In animals, the mechanical properties of tendons do not change from the end of growth to the senescence [23]. In humans, diameter and density of collagen tends to be reduced and the concentration of disorganised fibrils increases, probably as sequelae of lower functional demands [24].

We are aware of the limitations of our investigation. For example, this study reports on two relatively small populations of patients, with and without rupture to the quadriceps tendon. The older age of the control patients could be a bias, but it could further highlight the histological differences which we wanted to examine.

As we used haematoxylin and eosin without any additional sophisticated histochemical, immunohistochemical, and electron microscopic investigations, we have probably underestimated the incidence of degenerative changes in control tendons. However, the staining technique we used is readily and widely available, cost-effective, and easy to perform. Finally, we do not show evidence of the real incidence of quadriceps tendon abnormalities in adults.

Conclusion

Some changes to the tendon have been observed after rupture. Abnormal collagen distribution [11] and increased production of type III collagen fibres by tenocytes [25] are all features of the degenerative changes described in torn quadriceps tendons, with lower resistance to tensile forces and, consequently, increased risk of rupture.

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