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INFLAMMATORY CELL CONTENT OF SYNOVIAL FLUID AND MORPHOLOGICAL EVALUATION OF THE *VASTUS LATERALIS OBLIQUE* MUSCLE IN PATIENTS WITH ANTERIOR CRUCIATE LIGAMENT DISRUPTION

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ABSTRACT

The presence of inflammatory cells in the synovial fluid and the general aspect of the knee joint in patients (n=5) with chronic injury (from 1 to 15 years previously) of the anterior cruciate ligament (ACL) were evaluated. The morphology of the *vastus lateralis oblique* (VLO) muscle of the same leg was also examined. Synovial fluid and muscle biopsies were obtained during arthroscopy for ACL reconstruction. Synovial fluid was processed and stained with May-Grunwald-Giemsa. Sections of frozen muscles were stained with hematoxylin and eosin and processed to detect mATPase. Two patients showed erosive injury and fibrillation of the cartilaginous surfaces of the knee, while the other three patients had injured menisci. Cytological analysis of the synovial fluid showed only mononuclear cells in three patients and mononuclear and polymorphonuclear cells in another. The fifth patient had neither mono- nor polymorphonuclear cells. Although most of the VLO muscle fibers examined had a normal morphological aspect, bundles of fibers with centralized nuclei, atrophy and diameter irregularity were seen. These results show that in addition to the well known articular dysfunction associated with ACL injury, the synovial fluid also contains mono- and polymorphonuclear cells, and there are alterations in the VLO muscle.

Key words: Anterior cruciate ligament disruption, morphometry, quadriceps muscle, synovial fluid, *vastus lateralis oblique*

INTRODUCTION

The anterior cruciate ligament (ACL) of the knee has been extensively studied because of its important functional role in knee movements. Total or partial damage of the ACL can alter proprioception in the joint, and can modify the flexibility, coordination, postural control and equilibrium among muscles involved in knee movements [12]. Even following reconstruction of the ACL, the knee joint remains unstable and functionally altered. This chronic instability can lead to conditions such as arthrosis [8]. In addition, the acute knee effusion caused by this instability contributes to the degeneration of knee joint cartilage and alters the presence of cells in synovial fluid. An acute knee effusion also limits the range of knee movement, increases pain, and decreases muscle strength, all of which lead to functional disabilities [6].

Several studies have shown a significant decline in quadriceps muscle strength after ACL injury and reconstruction, when compared to the muscle of the opposite limb [3,10,15,18,29,33]. The maximal torque values of the quadriceps and hamstring muscles decrease when compared to those of the opposite limb, and for both muscles eccentric activity was more affected than concentric activity [13].

The skeletal muscle cross-sectional area has been related to the capacity of the muscle to produce isometric and isokinetic force [24,25]. Lorentzon *et al*. [23] found significant atrophy in the quadriceps, but not in hamstring muscles, in patients with chronic ACL injury. These authors also examined the muscle fibers of hamstrings, *vastus lateralis* and *vastus intermedius*, which generally had a normal morphology, but showed some type I and type II fibers with mild atrophy, with a predominance of type II fibers.

The morphological changes that occur in the quadriceps muscle fibers of patients with a damaged ACL have not been studied. Similarly relationship between the degree of knee joint inflammation and the morphology of the quadriceps muscle fibers has not been

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assessed. The *vastus lateralis* muscle has two morphologically and biomechanically distinct regions. The distal region of this muscle is responsible for the lateral stability of the patella [4] but its histopathology in knee disorders has not been studied. In this work, we examined the macroscopic aspects of the knee joint based on arthroscopy, and assessed the cytology of synovial fluid and the morphology of the *vastus lateralis oblique* muscle (VLO) in patients with chronic ACL injury.

MATERIAL AND METHODS

The patients provided informed consent prior to enrollment and the study was approved by the Ethics Committee for Human Studies of the Federal University of São Carlos (Resolution 196/96 of the National Health Committee).

Five men with chronic ACL injury were evaluated. Clinical examination showed that all of the patients had a positive pivot signal, with increased Lachman and anterior drawer tests. Surgery for ACL reconstruction by arthroscopy was indicated in all cases. Under anesthesia, the synovial fluid was sampled via the lateral articular interline, with the cavity being emptied when there was an intrarticular effusion. The joint was subsequently evaluated by artroscopy, and a biopsy of the VLO was obtained about 5 cm from the articular line in the lateral portion of the knee. The synovial fluid and skeletal muscle biopsy were immediately frozen in liquid nitrogen, and stored at –80°C.

The synovial fluid was centrifuged in a cyto-centrifuge (Fanem, Citospin, Brazil) for 5 min at 1,500 rpm and the slides then dried and stained with May-Grünwald-Giemsa stain prior to examination by light microscopy (Axioscope Carl Zeiss, Germany) to identify inflammatory mononuclear (MNC) and polymorphonuclear (PMN) cells.

Serial cross-sections (10 μm) of frozen VLO muscle were cut using a cryostat (Microm HE 505, Germany). Alternate serial cross-sections were stained with hematoxylin and eosin (HE) and toluidine blue for general morphological evaluation. Some sections were also stained for myosin ATPase activity (mATPase) after acid (pH 4.1) and alkaline (pH 9.8) pre-incubations. For each VLO muscle biopsy, the minimum diameter of 50 types I and type II muscle fibers was determined. The ratio of type I to II fibers was also calculated based on three microscopic fields chosen at random. All of the morphometric parameters were obtained using the image analysis software Image-Pro Plus (version 4.1 for Windows, Media Cybernetics).

RESULTS

Five men, 20 to 43 years old, with chronic ACL injury participated in this study. The duration of ACL injury ranged from 1 to 15 years (Table 1).

The VLO muscle samples showed predominantly polygonal fibers with normal morphology. There were no signs of muscle fiber necrosis or recent regeneration, and the connective tissue was normal. Some bundles of fibers with central nuclei were observed, especially in two patients, one who had suffered ACL injury 5 years previously (50% of the fibers with central nuclei) and another who had suffered ACL injury 15 years earlier (22% of the fibers with central nuclei) (Fig. 1A). In three patients who had suffered ACL injury 1.5, 7 and 15 years previously, the fibers had irregular diameters (Fig. 1B). Two VLO muscle biopsies obtained 1.5 and 15 years after ACL injury showed some angulated fibers.

Myosin ATPase reactions revealed type II fiber atrophy in four patients. The exception was a patient who had suffered ACL injury 1 year previously. Abun-

Table 1. Morphological and morphometrical evaluation of the synovial fluid, knee cartilage and *vastus lateralis oblique* muscle in patients with chronic ACL disruption.

Patients	1	2	3	4	5
Age (years)	20	31	32	25	43
Duration of ACL injury	1 year	1.5 years	5 years	7 years	15 years
Cartilage injury	Fibrillation	Fibrillation	Fibrillation Erosion	Fibrillation Erosion	Fibrillation
Type of ACL injury	Total	Total	Total	Total	Partial
Intra-articular effusion	Present	Present	Absent	Present	Present
Meniscus injury	MM+LM	MM	MM+LM	MM	MM
Cartilage color	White	White	White	White	White
Synovial fluid (ml)	3	13	Absent	3	5
Synovial fluid color	Pale yellow	Pale yellow	Absent	Turbid yellow	Turbid yellow
PMN	Small	Absent	Absent	Absent	Absent
MNC	Small	Small	Absent	Small	Small
Type I - min diameter (μm)	66.6 ± 11.7*	72.6 ± 15.3	54.5 ± 11.4	55.5 ± 14.7	67.7 ± 14.7
Type II - min diameter (μm)	73.6 ± 13.5	50.6 ± 15.8	62.9 ± 13.1	56.1 ± 13.2	59.7 ± 17.9
Type I:Type II Ratio	1:4.6	1:2.1	1:3.4	1:1.2	1:1.3

MM: medial *meniscus*; LM: lateral *meniscus*
PMN: polymorphonuclear cells; MNC: mononuclear cells;
*: Mean ± SD (standard deviation)

dant, undifferentiated fibers were observed in two patients who had suffered ACL injury for 1.5 and 15 years earlier (Fig. 1C,D). In general, type II fibers predominated in VLO (Table 1). Only one patient (15 years after ACL injury) showed the grouping of type II fibers. The mean values for the minimum diameters of type I and II fibers for each patient are shown in Table 1.

Intra-articular joint knee analysis showed that four patients had variable volumes of effusion in the bursa suprapatellar region (Table 1). All patients showed total ACL disruption, except for the patient who had suffered ACL injury 15 years earlier. All of the patients also had white cartilage and fibrillation in the medial condyle. Two patients (5 and 7 years after ACL injury) showed signs of cartilage erosions, classified as chondromalacea grade III. Medial *meniscus* injuries were observed in three patients (1, 5 and 15 years after ACL injury), two of whom also had related lateral *meniscus* injuries (Table 1).

The color of the synovial fluid changed from turbid yellow to pale yellow (Table 1). Clusters of inflammatory mononuclear cells were found in one patient (7 years after ACL injury) (Fig. 1E) while three other patients had isolated mononuclear cells in their synovial fluid (Fig. 1F). Polymorphonuclear cells were found in only one patient (1 year after ACL injury) (Fig. 1G), but were not the result of an inflammatory process.

DISCUSSION

The knee is a commonly injured joint. After injury to the ACL, there is often an acute knee effusion that limits the movement of the knee range, increases pain, and decreases muscle strength [20,26]. The presence of mono- and polymorphonuclear cells in the synovial fluid and the altered VLO morphology indicate that there may be morphological changes associated with the ACL damage.

The mean minimum diameters for type I and type II fibers observed here agree with the normal values for male subjects [9]. However, some isolated atrophic fibers were observed. Type II fibers may atrophy as a result of disuse, cachexia, and aging, whereas type I fibers are usually less affected. The preferential involvement of type II fibers in aging has been widely studied [14,17,19,21,32,34]. A minimal effusion can cause physiological changes in the knee joint that can inhibit the quadriceps [35] and result in an inability to fully use the *quadriceps femoris* muscle [27]. The type II fiber atrophy seen in four patients was assumed to be associated with the ACL ligament injury. On the other hand, other fibers could undergo compensatory hypertrophy through overuse, and this could explain the high proportion of fibers with central nuclei in the VLO of some patients.

The presence of angulated fibers is usually associated with aging [2,16,21] or with neurogenic factors that affect the fibers. Undifferentiated fibers are probably associated with muscle fiber reinnervation and regeneration [30]. In this study, associated angulated and undifferentiated fibers were seen in patients 31 and 43 years old. The oldest patient still showed type-grouping. In this case, the long interval since ligament injury probably interfered with the morphological alterations induced by aging, or could represent a degenerative disorder [37].

Mononuclear and polymorphonuclear leucocytes were rarely observed indicating that there was no joint inflammation. Platt [31] classified as normal synovial fluid with less than 50 cells/mm^3 and as inflammed fluid with 200 - 2000 cells/mm^3. Biasi *et al*. [5] showed that in dogs with ACL injury, the synovial fluid could have an effusion and inflammatory state up to 90 days after injury.

In addition to the presence of leucocytes and proteoglycan fragments, the color and volume of synovial fluid can be helpful in assessing joint health [22,28,36]. As shown here, the patients had different volume of synovial fluid, ranging from normal to within the limits for osteoarthritis. According to Fawthrop *et al*. [11], the volume of synovial fluid in normal humans ranges from 0.2 - 2 ml and from 1 – 50 ml in osteoarthritic subjects.

Patients 1 and 4 had 3 ml of effusion, which is normal, but patients 2 and 5 had 13 ml and 5 ml, respectively, indicating a possible arthritic condition. However, an increase in synovial fluid volume in knees with an injured ACL does not depend on the duration of injury. Knee instability from this type of injury results in frequent spraining that causes a new effusion [1] unrelated to the time since ligament injury. As shown here, subjects with different intervals since injury (1 to 15 years) had different volumes 3 - 13 ml of synovial fluid in the knee joint.

Fawthrop *et al*. [11] reported that normal synovial fluid should be clear and straw colored, but yellow or orange when inflammed or osteoarthritic. In this study, patients 1 and 2 had pale yellow fluid and subjects 4 and 5 (with longest time since injury) had tur-

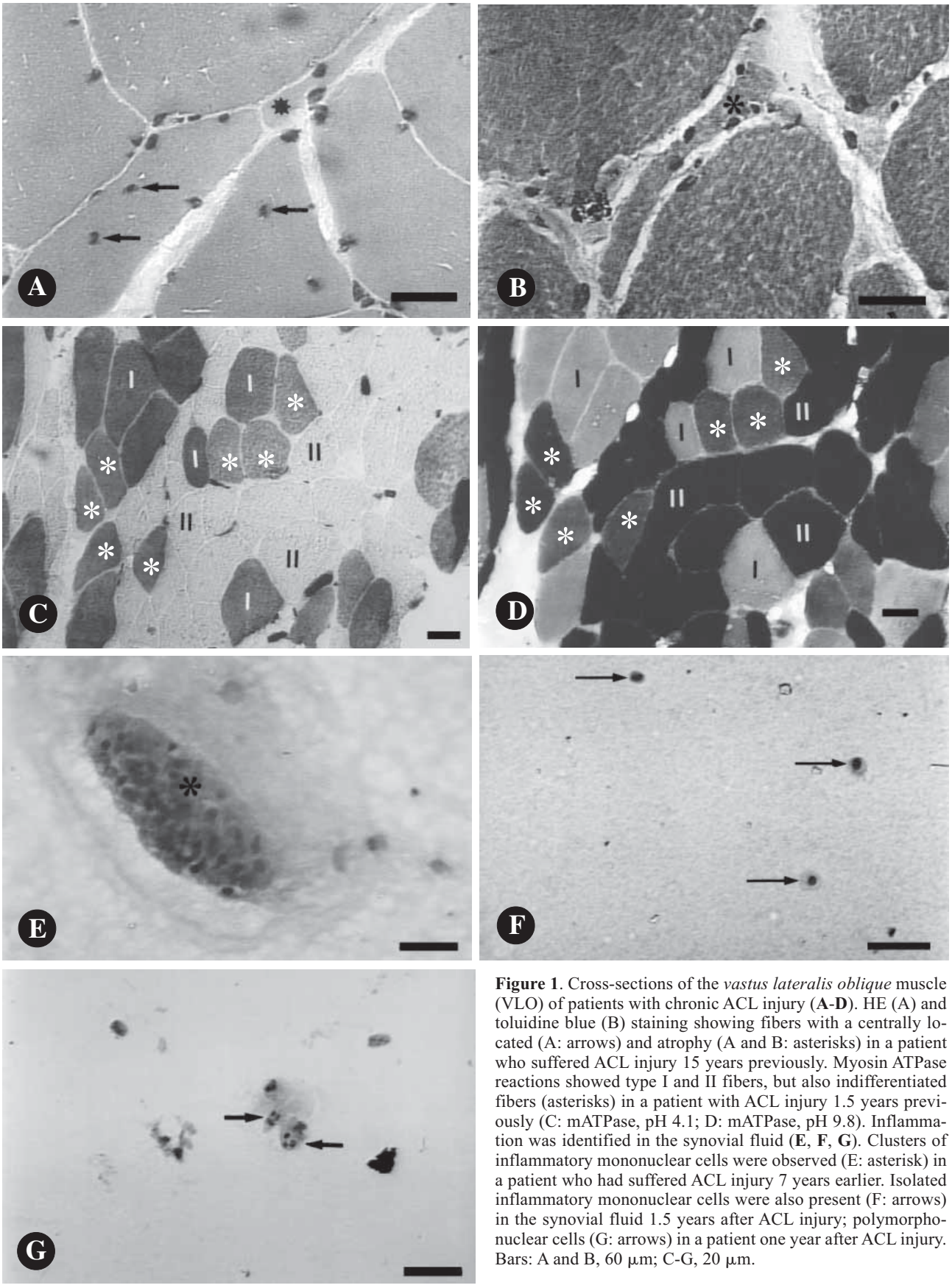

Figure 1. Cross-sections of the *vastus lateralis oblique* muscle (VLO) of patients with chronic ACL injury (**A**-**D**). HE (A) and toluidine blue (B) staining showing fibers with a centrally located (A: arrows) and atrophy (A and B: asterisks) in a patient who suffered ACL injury 15 years previously. Myosin ATPase reactions showed type I and II fibers, but also indifferentiated fibers (asterisks) in a patient with ACL injury 1.5 years previously (C: mATPase, pH 4.1; D: mATPase, pH 9.8). Inflammation was identified in the synovial fluid (**E**, **F**, **G**). Clusters of inflammatory mononuclear cells were observed (E: asterisk) in a patient who had suffered ACL injury 7 years earlier. Isolated inflammatory mononuclear cells were also present (F: arrows) in the synovial fluid 1.5 years after ACL injury; polymorphonuclear cells (G: arrows) in a patient one year after ACL injury. Bars: A and B, 60 μm; C-G, 20 μm.

bid yellow fluid. None of the patients had a normal synovial fluid color.

Degenerative changes in the articular cartilage, including fibrillation and erosion were seen in all subjects.

Cameron *et al.* [7] reported that patients with chronic ACL injury showed degenerative changes in their joints similar to those associated with osteoarthritis, even after joint reconstruction. Thus, subjects who had suffered longer from injury showed a condition that was similar to that of osteoarthritic joints.

Although the number of cases examined here was small, our findings suggest that: a) the time interval since ACL injury may not be directly related to the degree of inflammation in synovial fluid of the knee joint, b) the most significant alterations in VLO muscle fibers (irregular diameter, atrophied fibers and undifferentiated fibers) could be related to the increase in effusion or to the chronic state of the lesion, and c) ACL injury may alter the synovial fluid and VLO muscle to varying degrees, and this could contribute to loss of function in the articulation.

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