QUANTIFICATION OF NEURAL ELEMENTS IN POSTERIOR CRUCIATE LIGAMENT: COMPARISON BETWEEN HEALTHY KNEES AND WITH PRIMARY OSTEOARTHRITIS

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ABSTRACT

Objective: To quantify the neural elements in the posterior cruciate ligament (PCL) in healthy knees and with primary osteoarthrosis (OA). Methods: In two groups with OA, one of cadavers and another of individuals, the area of neural elements identified in histological sections of PCL with anti-S100 immunohistochemistry was quantified. Results: The overall mean area of the neural elements was 0.96% ± 0.67%, with the value in the cadaver group of 1.02% ± 0.67% and in the OA group of 0.80% ± 0.64%, with a significant statistically difference (p = 0.001). No correlation was observed between neural element quantification and the age of the individuals (p > 0.05). There was no difference in the quantification of neural elements between the sexes in the cadaver group (p = 0.766), but in the OA group there was a statistically significant reduction in males (p = 0.003). Also, in the osteoarthrosis group there was no difference in the quantification of neural elements in the knees with varus or valgus alignment (p = 0.847). Conclusion: There was a decrease in neural element quantification in PCL of individuals affected by OA in relation to non-arthritic individuals, with this quantification not related to age or with the axis of the lower limb. Level of Evidence III, Case control study.

Keywords: Posterior Cruciate Ligament. Mechanoreceptors. Nerve Tissue. Immunohistochemistry. Osteoarthritis.

INTRODUCTION

Mechanoreceptors are responsible for detecting the deformation of periarticular tissues and for the sensory coding of signals that inform about intrinsic and extrinsic joint forces. These signals offer the cognitive perception of the strength, positioning, movement, speed and direction to which the joint is submitted, contributing indispensably to joint homeoasis.1-3

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Osteoarthrosis (OA) is marked by capsuloligamentous laxity, loss of articular cartilage, bone deformity and limb misalignment, factors that strongly influence the loss of proprioceptive sensitivity. It is also observed the decline in proprioceptive capacity with the aging process, which also has an important relevance when observing the degenerative joint disease, characterized incident in older age groups.\textsuperscript{1,4} The joint replacement through arthroplasty has led to proprioceptive sensitivity at an intermediate level between the disease status and the normal, with the objective of restoring limb alignment, soft tissue balance and joint stability. The knees with more accurate proprioceptive capacity may be related to the load applied more physiologically, which is important in preventing the loosening and the wearing of arthroplasty implants.\textsuperscript{4-6}

Several studies examined the difference in proprioceptive capacity after the total knee arthroplasty (TKA) and compared the results of several joint reconstruction techniques, particularly the preservation or not of the posterior cruciate ligament (PCL), among other reasons, for believing that this structure has an important proprioceptive function;\textsuperscript{4,6} being demonstrated and studied the mechanoreceptors in the posterior cruciate ligament of the knee.\textsuperscript{2,3,7-12} However, benefits with the preservation of PCL have not been consistently observed. This may be related to a reduction in the population of mechanoreceptors with the advancing age and even faster in OA.\textsuperscript{2,8,11,13,14}

The aim of this study was to analyze the neural elements in PCL and to verify whether there is; a reduction in the quantification of neural elements in OA; a correlation with the age of the individuals investigated exists; a difference between males and females; and a difference between arthritic knees with varus or valgus alignment.

MATERIALS AND METHODS

This study, developed from July 2017 to December 2019, was conducted in accordance with the Helsinki Declaration of 1995, and was analyzed and approved by the Research Ethics Committee of the Medical School of Universidade Federal do Cariri (UFCA) (CAAE: 31115014.9.0000.5035). Two groups of individuals were studied, one consisting of fresh cadavers and another by patients with primary knee OA. All individuals studied or their legal guardians signed a Free and Informed Consent Form. In the cadaver group, both sexes were included, with no age restrictions, and 24 PCLs of 24 cadavers with no morbidity past in the joint were studied. The knee side was chosen by drawing lots. Macroscopic examination of the joint, as well as the medical, cadaveric and interrogation reports to family members or guardians were evaluated to make sure that there were no previous knee injuries. Cadavers with history or evidence of previous morbidity, inflammatory diseases and surgical past in the joint were excluded. The cadaver was positioned in ventral decubitus and through a posterior access to the knee, and the PCL was collected from the femoral insertion until the tibial insertion.

In the OA group, both sexes were included, with no age restrictions, and 14 PCLs of patients with primary OA, submitted to TKA with posterior stabilization, were studied. Patients with secondary OA and previous knee surgery were excluded. For collection during the TKA surgical procedure, after medial parapatellar arthroscopy and lateral dislocation of the patella, with the knee at maximum flexion, the osteophytes and anterior cruciate ligament (ACL) were resected, when present in the femoral intercondylar, the anteriorization of the tibia under the femur and proximal tibial cut, allowing the collection of PCL from its femoral insertion to tibial insertion.

The preliminary study with hematoxylin-eosin (HE) staining showed in all specimens evaluated ligament tissue with fibers and typical cells, and periligamentous and vasculonervous tissues of normal aspects. It did not show any pathological condition that could compromise the study of neural elements in the conditions proposed in the research.

For each ligament, a slide with anti-S100 immunohistochemistry (murine monoclonal antibody Clone 4C4.9 – ZETA Corporation) was prepared using the method as described by Kahn et al.\textsuperscript{15} and by Mihalko et al.\textsuperscript{16} following the processing and reaction according to the manufacturer’s protocol. The anti-S100 stained slide were analyzed by a Master of Prosthetics and Orthotics (M.P.O) and two doctors, professors of pathology, (R.J.V.M and L.T.M). An optical microscopy study was carried out with an increase of 100× (DI-115T microscope, DIGILAB Laboratory). Microscopic, and the visualization was performed throughout the entire tissue present in the slide. When anti-S100 stained neural tissue was identified in the field of vision of microscopy, the image was captured with a digital camera (model A59.4910, DIGILAB Laboratório) coupled to the microscope and the Micro Capture software (Ver6.9.12). The images were standardized in files with TIFF format. In each slide, the maximum number of images viewed was collected. All images were captured respecting the same standardization of magnification in microscopy, resolution and image size (Figure 1).\textsuperscript{17}

The images, standardized in size 1024 × 768 pixels with 96 dpi (dots per inch) RGB color, 1 layer, with a total of 786.432 pixels, were analyzed with the software GIMP 2.10.14 (GNOME Foundation). With this software, the “free hand” selection of the area corresponding the neural tissue observed in the image was performed (Figure 2) and the pixels referring to the selected area (Figure 3)\textsuperscript{17} were counted. The amount of pixels referring to neural tissues was recorded for each image as a percentage of the total pixels of the image.

![Figure 1. Histological sections of the PCL in case of the cadaver group (right) and OA (left). Staining with anti-S100 immunohistochemistry in which the presence of neural elements is evidenced in an increase of 100 times.](image-url)
The data were tabulated and analyzed with the software Statistical Package for the Social Sciences (IBM-SPSS, version 24). A p-value of 0.05 was set for statistical significance.

RESULTS

Table 1 describes the distribution of individuals by gender and age. There was no statistically significant difference between the groups in relation to age (p = 0.256) and gender (p = 0.088) of the individuals.

Table 1. Sample distribution by age and gender

| Group                  | Cadaver | Osteoarthrosis | p-value |
|------------------------|---------|----------------|---------|
| Age                    |         |                | 0.256   |
| Average                | 59.8    | 71.1           |         |
| Standard Deviation     | 24.4    | 8.4            |         |
| 95% CI                 | (49.5 – 70.1) | (62.2 – 75.9) |         |
| Minimum                | 13      | 57             |         |
| Median                 | 64.5    | 70.5           |         |
| Maximum                | 92      | 87             |         |
| Sex                    |         |                | 0.088   |
| Male                   | 13      | 3              |         |
| Female                 | 11      | 11             |         |

Note: (*) p < 0.05 Kruskal-Wallis test

A total of 374 microscopy fields were recorded, 276 in the cadaver group and 98 in the OA group. The overall mean area of neural elements was 0.96% ± 0.67% for both groups together, and in the cadaver group it was 1.02% ± 0.67% and in the OA group it was 0.80% ± 0.64%, with ± statistically significant reduction in the OA group (p = 0.001) (Table 2 and Figure 4).

Table 2. Quantification of the area of neural elements (%) per profile under analysis.

| Group                  | N  | Average | Deviation Standard | 95% CI | Minimum | Maximum | p-value |
|------------------------|----|---------|--------------------|--------|---------|---------|---------|
|                        |    |         |                    |        |         |         |         |
| General                | 374| 0.96    | 0.67               | 0.89   | 1.03    | 0.07    | 2.75    |
| Groups                 |    |         |                    |        |         |         |         |
| Cadaver                | 276| 1.02    | 0.67               | 0.94   | 1.10    | 0.09    | 2.75    |
| Osteoarthrosis         | 98 | 0.80    | 0.64               | 0.67   | 0.93    | 0.07    | 2.46    |
| Groups (Over 60 years) |    |         |                    |        |         |         |         |
| Cadaver                | 188| 1.04    | 0.66               | 0.94   | 1.13    | 0.13    | 2.75    |
| Osteoarthrosis         | 90 | 0.61    | 0.65               | 0.67   | 0.94    | 0.97    | 2.46    |
| Cadaver Group          |    |         |                    |        |         |         |         |
| Male                   | 153| 1.04    | 0.71               | 0.93   | 1.16    | 0.09    | 2.75    |
| Female                 | 123| 0.99    | 0.62               | 0.88   | 1.10    | 0.13    | 2.66    |
| Osteoarthrosis Group   |    |         |                    |        |         |         |         |
| Male                   | 23 | 0.45    | 0.32               | 0.31   | 0.59    | 0.07    | 1.13    |
| Female                 | 75 | 0.91    | 0.68               | 0.75   | 1.06    | 0.12    | 2.46    |
| Osteoarthrosis Group   |    |         |                    |        |         |         |         |
| Varus                  | 84 | 0.61    | 0.66               | 0.66   | 0.94    | 0.07    | 2.46    |
| Valgus                 | 14 | 0.77    | 0.55               | 0.45   | 1.08    | 0.21    | 1.82    |

*Kruskal-Wallis test
In order to minimize age bias in comparison, considering that the OA group is composed almost entirely of individuals over 60 years of age, groups including only individuals above this age group were compared. Nevertheless, the observation of a statistically significant reduction in the area of neural elements of the OA group was maintained (p = 0.001) (Table 2 and Figure 4). There was no correlation between the age of the individuals studied and the area of the neural elements either in the cadaver group (p = 0.521) or in the OA group (p = 0.079) (Figure 5).

In the cadaver group there was no statistically significant difference in the mean area of neural elements between the sexes (p = 0.766). In the OA group, there was a statistically significant reduction in the mean area of neural elements between the sexes (p = 0.003) (Table 2 and Figure 6).

In the OA group there was no statistically significant difference in the area of neural elements between the knees with varus and valgus alignment (p = 0.847) (Table 2).

**DISCUSSION**

In the present study, the overall mean of the area occupied by neural elements per field of microscopy was 0.96% ± 0.67. In healthy knees, this mean was 1.02% ± 0.67%, comparable to the values of 0.958% ± 0.13% found in the healthy knees studied by Franchi, Zaccherotti and Aglietti11; and at the value of 1% found by Schutte et al.18 in the anterior cruciate ligament of healthy knees. On the knees with OA, in the present study we found an average of 0.83% ± 0.67%, being higher than the average found by Franchi, Zaccherotti and Aglietti11 in their group of arthritic knees, which was 0.44% ± 0.132%. Zhang and Mihalko9 found quite different values of percentage of area occupied by neural elements in histological images studying two groups of knees with OA. In one group involving recovered TKA-CR PCLs, the average stained area in the cross-section studied by microscopy was 10.7% ± 5.1% in anti-S100 immunohistochemistry. In the second group, consisting of PCLs collected from TKA-PS, the average stained area was 11.1% ± 7% anti-S100. The authors did not observe a statistically significant difference in the area of the receptors between the groups. This large discrepancy in the areas in relation to the studies by Schutte et al., Franchi, Zaccherotti and Aglietti11 and the present study may be due to different methodology in microscopic and/or histomorphometric study.

In the comparison between groups, Del Valle et al.12 did not observe significant difference between the types of corpuscles, sizes and distribution between knees with OA and healthy. Franchi, Zaccherotti and Aglietti11 observed a statistically significant reduction in the area occupied by the neural network and mechanoreceptors in the PCL of the knees with OA in relation to normal knees (p = 0.001), findings similar to those of Çabuk et al.7 that although they did not find a difference between knee groups with OA and normal with regard to the number of Pacini corpuscles, they observed that the number of Ruffini corpuscles, Golgi, free nerve endings and total nerve endings was significantly lower in the OA group (p < 0.05). Marczack et al.3 observed in patients with primary OA a correlation between the severity of radiographic alterations and the presence of neural elements in PCL (p < 0.0001), so that samples with high degrees of degeneration had few receptors. They also observed a significant statistically decrease in the number of neural elements in patients with OA in relation to cadavers without joint disease (p < 0.0001). In the present study, a significant statistically reduction in the area of neural elements was observed in the OA group in relation to the cadaver group (p = 0.001), even with the comparison between groups with paired ages above 60 years (p = 0.001), reinforcing the role of OA in reducing the quantification of neural elements in PCL, since both groups were equated to the same age group.

The influence of age on the characteristics of neural elements in PCL has been verified in some studies. Moreno et al.7 who studied 15 PCLs of cadavers without joint morbidity, did not observe a correlation between the age of the cadaver and the total number of mechanoreceptors. A finding similar to that of Martins, Camanhão and Rodrigues,10 who identified immunomarkers for neural structures in 67.5% of the 34 PCLs of patients with primary OA, but did not observe a correlation between the age of the patients and the presence of neural elements. Colleoni et al.8,20 studying a male population of 19 cadavers without joint morbidity, did not observe a significant relationship between the total number of mechanoreceptors and age in the femoral or tibial portions of the PCL. However, in the tibial insertion, they observed a statistically
significant inverse correlation between age and the number of type I and type III mechanoreceptors, indicating that the older the age, the lower the number of these types of receptors in the tibial portion. In the present study, it was observed that there was no correlation between the mean area of neural elements and the age of the individuals evaluated both in the cadaver group and in the OA group. Thus, it is evident in the sample studied that the aging process was not related to a reduction in the quantification of neural elements in PCL.

Moreno et al.7 and Colleoni et al.8 point out that they did not find data in the literature demonstrating or suggesting the need for a comparative study in relation to gender, thus, they included only male individuals in an attempt to standardize their samples. In their work, Martins, Camanho and Rodrigues10 observed that there was no association between sex and the presence of neural elements. In the present study, it was observed that in the knees of the cadaver group there was no difference in the quantification of the area of neural elements between the sexes (p = 0.766). However, in the OA group, there was a lower quantification of the area of neural elements in males than in females (p = 0.003). It is evident, that although the literature does not pay attention to the difference in the quantification of neural elements in knee PCL between the sexes, this variable is necessary in order to better understand the characteristics of neural structures in knee PCL. This need reinforces the discussion about the differences between the sexes not only in some aspects of proprioception, but also in studies evaluating the knee joint.19,20

Evaluating the neural elements and their behavior in relation to knee alignment in varus or valgus, Martins, Camanho and Rodrigues10 observed that neural structures were more frequent in varus knees (77%) than in the valgus (50%), statistically significant difference (p = 0.048). In opposition, in the present study, no statistically significant difference was observed in the quantification of neural elements between the varus and valgus knees (p = 0.847). No other study was identified that evaluated limb alignment and neural elements in PCL.

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