Cross-sectional Study

Mechanoreceptor analysis on femoral and tibial site remnant of anterior cruciate ligament injury using immunohistochemistry

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ABSTRACT

Introductions: More than 50% of knee ligament injuries are anterior cruciate ligament (ACL) injuries. The injury can lead to instability and osteochondral damage which in turn leads to early osteoarthritis. ACL remnant contains sensory nerve cells and mechanoreceptors which are useful for reinnervation of the graft and maintaining the knee stability. However, ACL remnant preservation can interfere the visualization during surgery. The number of mechanoreceptors in the ACL remnant of the tibia and femur have to be determined to help the surgeon get better visualization and at the same time preserve the mechanoreceptors in the ACL remnant during reconstruction.

Methods: This study aims to evaluate semi-quantitatively the expression of mechanoreceptors in ACL remnant in the tibia and femur using immunohistochemistry. From January to April 2021, 10 femoral and 10 tibial remnants was obtained from ACL injury patient who received arthroscopic ACL reconstruction. Both of them were analysed using immunohistochemistry with S100 and NFL antibodies. The type of remnant was recorded and the expression of the mechanoreceptor was observed under the microscope.

Results: The most common type of ACL remnant recorded were type 2 (50%), followed by type 1 (40%) and type 4 (10%). There were no significant differences in the expression of mechanoreceptors between femoral remnant and tibial remnant (p = 0.45 and p = 0.134).

Conclusions: No difference in the expression of mechanoreceptors of femoral and tibial ACL remnant. Preservation of both femoral and tibial remnants is important in ACL reconstruction surgery.

1. Introduction

Injury to the anterior cruciate ligament (ACL) is a common injury that occurred in more than 50% of overall knee injuries. The incidence of ACL injury is 30–78 cases per 100,000 people [1]. This injury is also often accompanied by other knee injuries such as the medial collateral ligament (MCL) (19–38%) and lateral collateral ligament (LCL) (20–45%) or medial meniscus (0–28%) [2]. Injury to the ACL causes acute pain, hemarthrosis, and instability of the knee [3]. In a study of athletes who continued their activities with injuries of the ACL, several episodes of instability occurred and lead to the meniscus and osteochondral injury which also ultimately led to early osteoarthritis [4]. The incidence of osteochondral damage occurs in 21–31% of cases [4,5].

Surgical management is the recommended option to restore joint stability, especially for young patients with high-level activity, or athletes who wanted to return to sports competition [3,6]. Long-term studies have shown an increase in the degree of damage to the meniscus and joint cartilage in cases of ACL with delayed reconstruction measures [7]. However, in a randomized control trial (RCT) involving young patients comparing early reconstruction (10 weeks after injury) with delayed reconstruction, there was no significant difference between the two groups [8]. Nevertheless, surgical management remains a recommendation as initial therapy in professional athletes [3].

Several factors could be associated with the failure of ACL reconstruction. It includes graft impingement, graft laxity, and inaccurate tunnel of the tibia and femur which produce the histological and biomechanical characteristics of the original ligament [9]. In addition, 15%–25% of patients undergoing ACL reconstruction continue to...
experience knee joint pain and instability [10]. In some cases of ACL reconstruction surgery, a surgeon could find a remained tissue of ACL called the remnants. Remnants can be left alone or removed. If this remnant is left, it can interfere with visualization during operation so it will have an impact on the final quality of the operation [9,11]. However, remnant preservation is also believed to positively impact the outcome of ACL reconstruction. This study aimed to evaluate the expression mechanoreceptor at ACL remnant both on the tibia and femoral site. This study has been presented in line with the STROCSS criteria [12].

2. Methods

This was a descriptive study performed on human subjects with ACL injuries. This study was conducted based on ethical clearance by the ethics committee of Prof. Dr. R. Soeharso Orthopedic Hospital Surakarta. The study aims to evaluate the expression of mechanoreceptors semi-quantitatively in the ACL remnant both on the tibia and femur site using immunohistochemistry. Patients who meet the requirements were included as a sample while those who do not meet the requirements were excluded. The inclusion criteria are as follows: patient with an ACL tear, no previous surgical intervention, no general comorbidities, willing to undergo surgery, and willing to be a research sample. Exclusion criteria include the history of previous surgery on the knee area, age over 50 years old, rheumatoid arthritis or other knee inflammation, partial ACL injury not needed surgery, knee osteoarthritis more than grade I, infection, and refusal to be a research sample.

The sample size used was the purposive sampling method. This technique is carried out by researchers who select patients according to inclusion and exclusion criteria. An immunohistochemistry examination was performed at the Laboratory of Pathology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta. Monoclonal antibodies of NFP can characterize and colour-specific the cylindrical axons of proprioceptive fibers. Monoclonal antibodies against S-100 specific protein to mark or stain the Schwann cells and provide staining for nerve fiber endings such as Ruffini, Pacini, and free nerve endings under a microscope (10x and 40× magnification) [13]. Mechanoreceptors will be assessed based on their morphology and the expression of mechanoreceptors was measured in percentage of the total number of cells examined and the number of cells that stained positive with immunohistochemical markers. Assessment of immunohistochemical staining will use the pathological visual score (PVS) method with a range of 10%–100% [14, 15]. The obtained data were analysed statistically using SPSS® 25 (IBM®) software to see a difference between the expression of mechanoreceptors in the femoral remnant and tibia remnant. The degree of significance used was p < 0.05.

3. Results

A total of 13 patients underwent data collection, physical examination, and additional examinations in the form of radiographs and MRI. All patients received arthroscopic ACL reconstruction. Among the 13 patients, three patients met the exclusion criteria due to the ACL injury was found to be partial and no reconstruction procedure was needed. There were 10 patients and 20 remnant samples that were included in the pathology study. Among all 10 patients, 9 (90%) were male and only 1 (10%) was female. The age range of the youngest patients was 17 years and the oldest was 36 years with an average age of 23 ± 5.53 years. The side of the injured knee was equal between the right and left sides. All patients underwent ACL reconstruction with an all-inside technique. The onset of injury to the surgical procedure was varied. It ranges from 3 months to 3 years. A total of 4 (40%) patients had a history of sport injury while playing soccer, 1 (10%) patient had an injury during cycling, 4 (40%) patients had a history of slipping in the bathroom, and 1 (10%) patient experienced motorcycle accident.

The chief complaints of patients include pain and instability in 3
patients (30%) and instability only in 7 patients (70%). All patients had no systemic comorbidity. Physical examination of the knee revealed all patients had positive results on the anterior drawer test and Lachman test. Nine patients were diagnosed with isolated ACL rupture and one patient was diagnosed with concomitant MCL injury. All patients received ACL reconstruction with an all-inside technique and remnant preservation.

3.1. Intraoperative findings

During the operation, all patients had remnants of ACL in both of femur and tibia sites. Therefore the total obtained samples include 10 remnants of the femur and 10 remnants of the tibia. Each sample had the size of around 1 × 2 mm. The most common type of ACL remnants classification was Crain Type 2, which is the type that bridges the tibia and attaches to the PCL (Fig. 1a) in as many as 5 samples (50%). Type 1 is the type that bridges the tibia and attaches to the roof of the intercondylar notch (Fig. 1b) as many as 4 samples (40%) and type 4 does not bridge (Fig. 1c) as many as 1 sample (10%).

3.2. Immunohistochemistry staining

All samples were stained with immunohistochemistry with the use of S100 and NFP monoclonal antibodies. The pathologic evaluations were carried out by a consultant pathologist using the pathologist visual score (PVS) [13]. The results of the staining can be seen in Figs. 2 and 3. The immunohistochemistry staining showed 16 samples (80%) had mechanoreceptors marked by positive staining and as many as 4 samples (20%) did not have marked mechanoreceptors.

3.3. Statistical analysis

A Shapiro-Wilk test was conducted to determine the normality of the data. The normality test showed that the data distribution was abnormal, namely a significance value of 0.001 ($p < 0.05$) for staining with S100 remnant femur and tibia. A significance value of 0.002 ($p < 0.05$) on staining with NFP remnant femur and insignificance of 0.13 ($p > 0.05$). Therefore, a non-parametric test was performed with the Mann-Whitney test. The Mann-Whitney test showed that there was no difference between the remnant femur and tibia groups on the expression of mechanoreceptors using the S100 antibody ($p$ value > 0.45). Similar findings were also obtained in staining with NFP antibodies which resulted in no difference ($p$-value 0.13) See Table 1.

4. Discussions

In this study, the highest percentage of ACL remnant classification was type 2 (50%), where the type in which the remnant ACL attaches or bridges, creating scarring on the PCL. The results of this study are in accordance with the research on the morphology of remnant ACLs conducted by Crain et al. [16]. They found that the type of remnant ACL that adheres the most and creates scar tissue in the ACL (38%). This classification is in fact related to the biomechanics and stability of the knee joint according to research from Nakase et al. [17] that the remnant ACL that attaches to or bridges the PCL (type 2) is more stable against anterior translational and rotational forces compared to other types.
evaluated at an angle of 30°, 60°, and 90°, followed by type 1, and type 3. With the influence of the morphology of the ACL remnant on the stability and biomechanics of the knee joint, this variation in the type of ACL remnant needs to be carefully evaluated by an orthopedic surgeon when performing arthroscopic procedures to determine the next treatment for this remnant.

In this study, all of the patients still had ACL remnant at the insertion in the femur. The existence of this remnant ACL has the potential to accelerate graft remodeling of bone and tendon healing, as well as maintain knee joint stability with the role of its mechanoreceptors to carry out neo-innervation [18]. Many studies of histology and the potential of ACL remnant in humans have been carried out. A previous study by Lee et al. [19] evaluated the histology and cytology of ACL remnant based on their location. They discuss the potential for differentiation in the distal third of the remnant ACL (10 mm from the insertion in the tibia), such as the medial (10–20 mm). From the insertion in the tibia), and the proximal third (above 20 mm from the insertion in the tibia) [18]. The distal third of ACL remnant tends to have more cells with chondrogenic differentiation and high CD34 expression, while the middle third of remnant ACLs tend to have more cells with osteogenic differentiation and fibrotic ligament differentiation [19].

Another study conducted by Dhillon et al. [13] in patients with ruptured ACL reported that there was no significant difference between the tibia and femur. Although the previous study has different method of evaluation by calculating mechanoreceptors and assessing their morphology. In our recent study, we utilized a more standardized method with the use of a scoring system/scale. The results of the current research on the identification of mechanoreceptors in ACL remnant using immunohistochemical techniques showed that there were no significant differences between the femur and tibia site, even with thin slices and the cutting distance measured according to the span of the mechanoreceptors.

This study showed that the proprioceptive potential was found in ACL remnant as indicated by the presence of mechanoreceptors in 16 samples (80%) of 20 samples, both identified by immunohistochemical staining of S100 and NFP antibodies. Dhillon et al. [13] reported that there is a significant relationship between the length of the stump and morphology with mechanoreceptor immunoreactivity. In addition, there is no relationship between age, gender, and time of injury with the number of mechanoreceptors. However, they found that there is a significant relationship between the time of injury and the size of the ACL stump remnant [13,20].

In this study, the mechanoreceptor presents in both femur and tibial remnants in the same amount of expression. The findings of this study may support that preservation of remnant are important on both the femoral and tibial side. The attending surgeon may choose and estimate how much remnants will be removed according to the length of the remnants between the femur or tibia while maintaining the

| Monoclonal antibody | Shapiro-Wilk normality test | Mann-Whitney difference test |
|---------------------|---------------------------|------------------------------|
|                     | Femur | Tibia | Femur | Tibia |
| S100                | 0.001 | 0.001 | 0.45  |
| NFP                 | 0.002 | 0.132 | 0.13  |

Fig. 3. Mechanoreceptors showed in immunohistochemistry staining with NFP monoclonal antibody and the reading of using the pathologist visual score (a) 0%, (b) 50%, (c) 80%, (d) 100%.
The proprioceptive function of ACL. This study would be the base data for future work on the evaluation of mechanoreceptors in a case of reconstructed ACL injury. Knowing the expression of mechanoreceptors in ACL graft after the reconstruction may help the surgeon to individualize the rehabilitation program to improve the patient’s recovery after surgery. Our study has several limitations. First, our study included a limited number of samples due to ethical issues. Second, many other cell components in this ACL remnant also play a role in maintaining knee joint function after reconstruction of ACL which have not been evaluated. Thus, further research is needed in the form of analysis of mechanoreceptors not only in ACL remnants, but also in ACL-grafts after ACL reconstruction surgery.

5. Conclusions

No difference in the expression of mechanoreceptors of femoral and tibial ACL remnant. Preservation of both femoral and tibial remnants is important in ACL reconstruction surgery.

Ethical approval

The paper has been approved by Hospital Ethical committee.

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None to disclose.

Author contributions

Denny Adriansyah: Data collection, analysis, writing the paper.
Asep Santoso: Perform surgery, writing the paper.
Tangkas Sibarani: Concept and final analysis.

Consent

Informed consent to the patients has been performed prior to this publication.

Registration of research studies

1. Name of the registry: Researchregistry.com
2. Unique identifying number or registration ID: researchregistry7923
3. Hyperlink to your specific registration (must be publicly accessible and will be checked):

Guarantor

Asep Santoso, MD.

Declaration of competing interest

None to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.amsu.2022.103849.

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