A Histological Study of the Medial Meniscus Posterior Root Tibial Insertion

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Running head: Medial meniscus posterior root tibial insertion

Abstract

Purpose/Aim: Meniscal roots are important anchoring structures that allow normal meniscal function. Disruption of the medial meniscus posterior root leads to altered biomechanics, resulting in degeneration of knee cartilage. Although the transtibial pullout repair technique is recommended to restore medial meniscus function in patients with medial meniscus posterior root tear, correct placement of the tibial tunnel seems to be critical. The aim of this study was to evaluate the tibial attachment of the medial meniscus posterior insertion using a histological analysis.

Materials and Methods: Tibial surface samples were obtained from the osteoarthritic knees of seven patients who underwent total knee arthroplasty. Samples were divided into four regions (2.0 mm thickness) in a medial-to-lateral direction. Histological measurements of the medial meniscus posterior insertion were performed using safranin O-stained and type II collagen deposition sagittal sections. Furthermore, the tibial attachment area of the medial meniscus posterior insertion was equally divided into an anterior, central, and posterior area.

Results: Medial meniscus posterior insertion has its broadest connection with the tibial articular surface in line with the medial intercondylar tubercle. Deposition of safranin O-stained proteoglycan and relative type II collagen-staining density was higher in the anterior one-third of the medial meniscus posterior insertion than in the central-to-posterior area.

Conclusions: The structure of the medial meniscus posterior insertion was mainly localized at the anterior one-third. Our results can help to create an accurate tibial bone tunnel within the dense posterior insertion during transtibial pullout repair of medial meniscus posterior root tear.

Key words: Medial meniscus posterior insertion, histological analysis, transtibial pullout repair, medial meniscus posterior root tear, knee osteoarthritis

Introduction

The medial meniscus (MM) functions to stabilize the knee and distribute load within the joint. In particular, the posterior root of the MM plays an important role in anchoring the meniscus. Several reports have suggested that damage to the posterior root of the MM causes failure of the hoop structure of the meniscus, which negatively affects load distribution and increases tibiofemoral contact pressure, eventually leading to osteoarthritis of the knee (1-3).

Previously reported therapies for MM posterior root tear include arthroscopic transtibial pullout repair, all-inside meniscal repair, and conservative therapy (4-6). Recently, arthroscopic transtibial pullout repair was reported to be more successful than conservative treatments (7,8). For this reason, the importance of early diagnosis of MM posterior root tear has recently been recognized,

with early surgery recommended in suitable patients, when feasible (2,9). If the MM posterior root tear is repaired, the tibiofemoral contact surface area becomes greater, subsequently decreasing contact pressure (10), which in turn helps to control cartilaginous degeneration and prevent progression of knee osteoarthritis.

Conversely, if MM posterior root tear therapy does not sufficiently repair the meniscal root, the meniscus cannot regain its prior level of function. LaPrade et al. reported that insufficient repair of the meniscus results in similar functional outcomes as total resection of the meniscus (10). In transtibial pullout repair, the correct placement of tibial burr holes is critical and is as important as meniscal allograft transplantation.

Several past anatomical studies have reported on the position of the tibial attachment of the MM posterior insertion (11-13). LaPrade et al. reported that the MM posterior insertion is located 9.6 mm from the medial tibial eminence apex and 8.2 mm from the posterior cruciate ligament (PCL). Other previous studies reported the histological analysis of the meniscal tibial insertion. However, they described the structure of the meniscal tibial insertion (14, 23). Few studies have focused on the position of the MM posterior insertion using histological analysis. Therefore, the aim of this study was to evaluate the tibial attachment position of the MM posterior insertion using histological analysis. We hypothesized that the tight connection of the MM posterior insertion is located in line

with the medial tibial eminence on the anterior aspect of the attachment area based on previous anatomical reports.

Materials and Methods

Specimen preparation

This study was approved by our Institutional Review Board (Okayama University No. 1608-019), and written informed consent was obtained from all patients. Tissue samples were obtained from the osteoarthritic knees of seven patients who underwent total knee arthroplasty between April 2016 and February 2017. Among the study participants, three were men and four were women, with a mean age of 75 years (range, 67–86 years). MM posterior insertion samples with bony avulsions, degenerated MM posterior insertion, severe varus deformity, and osteophytosis of the medial tibial eminence were excluded. The anterior cruciate ligament (ACL) and PCL were detached from their respective femoral footprints during total knee arthroplasty. Osteotomy of the tibial surface was performed using a System 6 sagittal saw (6208-000-000 Stryker, Kalamazoo, MI) without damaging the tibial insertions of the MM and PCL. The samples were fixed in 10% formalin solution and then defatted in ethanol. Then, the samples were decalcified in 20% ethylenediaminetetraacetic acid (EDTA) solution (DOJINDO, Osaka, Japan) for two months. Tissue

samples were divided into four regions (slices 1–4; 2.0 mm thickness) (Figure 1). Initially, we divided along the medial intercondylar tubercle using a razor (Figure 1, slice 2). Other slices were then divided in parallel with each slice measuring 2.0-mm by 2.0-mm. The samples were embedded in paraffin using an automated tissue processor (RH-12 SAKURA Rotary, Tokyo, Japan). The paraffin embedded blocks were sliced on a microtome (Yamato-kouki, TU-213-SN) to obtain 6.0-μm slices. We analyzed the medial side of each block in the medial-lateral direction of the tibia. The specimens were analyzed using a microscopy (OLYMPUS BX50-33, Tokyo, Japan) and software (OLYMPUS cellSens standard 1.18, Tokyo, Japan) in our study.

Histological and immunohistological analysis

Histological assessments of the MM posterior insertion sagittal sections (slices 1–4, 6 μm thickness) were performed using safranin O-staining as previously described (15). For immunostaining, paraffin sections were deparaffined and 0.1% proteinase K (QIAGEN, Tokyo, Japan) was used for antigen retrieval of the sections (for 20 min at room temperature, Sigma, St. Louis, MO). We performed blocking endogenous peroxidase using 3% hydrogen peroxide (NACALAI TASQUE, Kyoto, Japan). After blocking in 3% BSA in PBS, the slides were incubated with mouse anti-type II collagen antibody (1:100, MP Biomedicals, Solon, OH) at 4ºC overnight.

After the overnight treatment, we used Histofine Simple Stain MAX PO (NICHIREI, Tokyo, Japan) as the second antibody for 60 minutes. Color was developed using Histofine diaminobenzidine solution (NICHIREI, Tokyo, Japan). Hematoxylin was used as a counterstain. For the negative control, all reagents, except for the primary antibody, were used. To quantify the signal densities of safranin O and type II collagen depositions, the processed image was loaded into Image J 1.31 (available as freeware from https://imagej.nih.gov/ij/) (16). The tibial attachment area of the MM posterior insertion was equally divided into an anterior, central, and posterior area in slice 2. Then, three different regions were selected within the calcified fibrocartilaginous layer of each area (200 × 200 pixels). Then, the image was divided colors into three colors (red (R), blue (B), green (G)) using the menu option Image-> Color->Split Channels (17). The proportion of the red color to all of the colors primarily represented the safranin O binding and was calculated using the equation $r = R/(R^2 + B^2 + G^2)^{1/2}$. The higher the *r* value represented the safranin O density. The ratio of the type II collagen staining density was also determined. The previously mentioned divided three colors was used to analyze the ratio of the type II collagen staining density. The proportion of the blue color to all of the colors primarily represented the interstitial tissue and cells. The proportion of the green color to all of the colors represented the cells. The ratio of the type II collagen staining density was calculated by subtracting a green area from the blue area. The mean values were calculated in each

of the three regions. We compared the mean values of each area in slice 2. The number of cells in each area was also evaluated in slice 2 with safranin O staining (400 μm × 400 μm).

Two orthopedic surgeons performed the analyses for collagen signal density in a blinded manner. Both intra- and interobserver reliabilities were good (ICC > 0.90) for each measurement.

Measurements of the MM posterior insertion length

Histological measurements of the MM posterior insertion length were performed using safranin O-stained slides (slices 1-4). The tibial attachment of the MM posterior insertion was determined as the width of ligamentous fibers stained by safranin O dye. To determine the medial edge of the MM posterior insertion, we used an MM-bone crossing point by distinguishing the medial border of the MM insertion fibers. We also used an MM-bone crossing point by distinguishing the lateral border of the MM insertion fibers to determine the lateral edge of the MM posterior insertion. The center of the MM posterior insertion was determined as center of the anterior and posterior edges in slice 2. The measurement of the MM posterior insertion center from the medial tibial eminence apex was performed.

Two orthopedic surgeons performed histological measurements of each length in a blinded manner. Each observer performed each measurement twice, at least two weeks apart. Correlation

analysis of the prominence ratio calculated based on parameters measured by two independent observers revealed an interclass correlation coefficient of 0.90 and an intraclass correlation coefficient of 0.92. This indicated that the prominence ratio measurements were highly reliable, regardless of the observer involved and the timing of observation.

Statistical analysis

All data are presented as means ± standard deviations. Differences between groups were compared using the one-way analysis of variance (ANOVA) with post-hoc test (Tukey-Kramer Honest Significant Difference test). Statistical significance was set at $P < 0.05$.

All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, EZR is a modified version of R commander designed to add statistical functions frequently used in biostatistics (18).

Results

There was a significant difference in the length of the MM posterior insertion attachment in each slice ($P < 0.05$). The safranin O-stained MM posterior insertion attachment was observed in slice 1, slice 2, and slice 3, but not in slice 4. The mean MM posterior insertion length was 2.1 ± 0.6

mm in slice 1, 7.7 ± 0.9 mm in slice 2, and 5.5 ± 1.3 mm in slice 3 (Figure 2). The widest MM posterior insertion was observed in slice 2.

The values of safranin O-staining density in each sample were calculated as follows: anterior area 1.0 ± 0.03; central area 0.9 ± 0.03; posterior area 0.86 ± 0.03. Safranin O-staining density of the anterior aspect was higher than that of the central and posterior areas in slice 2 (Figure 3e). There was no significant difference in safranin O-staining density between the central and posterior areas. The mean cell numbers were 30.0 ± 2.0 per square 400 μm in the anterior area, 21.0 ± 1.0 in the central area, and 16.7 ± 2.1 in the posterior area. The cells in the anterior area were greater in number than the cells in the central area and posterior area in slice 2 (Figure 3f). There was no significant difference in cell number between the central and posterior areas. Relative type II collagen-staining density of the anterior area was higher than that of the central area and posterior area in slice 2 (Figure 4). There was a significant difference in relative type II collagen-staining density between each area.

Histological measurement of the distance from the MM posterior insertion center to the medial tibial eminence apex was performed using safranin O-stained slides in slice 2. The mean distance was 9.9 ± 1.3 mm (Figure 2).

Discussion

Our study results indicated that the MM posterior insertion has its broadest connection with the tibial articular surface in line with the medial tibial eminence, and the strong fibrous enthesis composing the structure of the MM posterior insertion is located at the anterior aspect of the attachment area. Our histological analysis showed that the MM posterior insertion inserts on the bone in the regions are 2.0 mm medial to, and 2.0 mm lateral to, a line drawn from the medial tibial eminence apex. The insertion within the medial 2.0 mm region was 2.1 ± 0.6 mm, and the insertion within the lateral 2.0 mm was 5.5 ± 1.3 mm. Both attachments had significantly decreased attachment length compared with the attachment located in line with the medial tibial eminence, which was 7.7 ± 0.9 mm (Figure 2).

Several studies have reported the location of the MM posterior insertion. In an anatomical study by Johannsen et al., the MM posterior insertion was found to be located 0.7 ± 0.4 mm lateral to the medial tibial eminence line (19,20). Furumatsu et al. reported that the MM posterior insertion is located at a point 80% of the occipitofrontal diameter anteriorly and 40% of the transverse diameter medially from the tibial articular surface (21). In another study, James et al. used X-ray analysis and determined that the MM posterior insertion was 2.3 mm lateral to the medial tibial eminence line and 12.7 mm medial to the lateral tibial eminence line (22). Similar to previous

anatomical studies, our study confirmed that the lengthiest portion of the MM posterior insertion was centered on the medial tibial eminence line.

In our study, we found that the safranin O and type II collagen staining density were higher in the anterior area of the MM posterior insertion compared with the densities in the central and posterior areas (Figure 3e, 4d). These results suggest that the strong fibrous enthesis is most developed in the anterior part of the MM posterior insertion attachment area and the area that received the strongest tension (23). In transtibial pullout repair for the MM posterior root tear, the correct placement of the tibial tunnel seems to be critical. LaPrade et al. reported that anatomic repair exhibited significantly lower contact pressure than non-anatomic repair with a difference of -54% relative to the intact contact pressure (10). In the study of autograft transplantation, Sekaran et al. reported that placement of the posterior horn tunnel in the non-anatomic posterior location caused a significant posterior shift in the centroid of the contact area and a significant increase in the tibiofemoral contact pressure (24). We consider our results obtained from histological analyses useful in deciding the accurate position of the tibial tunnel for orthopedic surgeons. Non-anatomic tibial tunnel placements, such as large tunnel aperture on the medial tibial plateau and unfavorable guide setting at the medial side of the PCL, will be avoided. An accurate tibial tunnel placement at the anterior one third of the MM posterior root attachment may improve postoperative clinical

outcomes following MM posterior root tear pullout repair by restoring the tibiofemoral contact pressure.

There were several limitations in this study. First, most samples in our study were obtained from elderly patients who had varus arthritic knees. Minor damages of the menisci are often observed in osteoarthritic knees. In our study, we excluded the samples with degenerated MM posterior insertion. However, minor meniscal damages may influence our results. Further investigations will be required to determine the optimum location of the tibial tunnel using tissue samples derived from patients with normal knee alignments. Second, we included a small number of patients in this study. Our results will need to be confirmed by examining a larger sample group. Third, although the histological analysis of the MM posterior insertion was not performed by an expert pathologist, orthopedic surgeons who were trained to evaluate histological findings of the enthesis and meniscus assessed the MM posterior insertion in this study. Finally, the clinical results following the MM re-insertion technique have not been verified in this study. Further investigations will be required to understand the clinical efficacy of tibial tunnel placement at the anterior one-third within the MM posterior root attachment for pullout repair of the MM.

Conclusions

This histological study showed that MM posterior insertion was located on the extended line of the medial tubercle and the insertional structure of the MM posterior insertion was mainly localized at the anterior aspect of the MM posterior root attachment. We recommend that the surgeon be conscious of the anterior aspect of the MM posterior root attachment area during tunnel creation intraoperatively.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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on the tibial plateau. Am J Sports Med. 2002;30(1):74–82.

Figure legends

Figure 1. The MM posterior insertion. (a) Top view of the tibial surface of the right knee.

Tissue samples are divided into four segments by dashed lines (slices 1-4). Red point, medial tibial eminence apex. LM, lateral meniscus. (b-e) Prepared segments. Red point, medial tibial eminence apex. Black dashed areas, the MM. Bars, 1 cm.

Figure 2. (a-d) Safranin O-stained specimens (a, slice 1; b, slice 2; c, slice 3; d, slice 4)

Red point, medial tibial eminence apex. Black dashed areas, MM. White dashed line, MM posterior insertion. White broken line, PCL margin. Bars, 5 mm. (e) The length of the MM posterior insertion in each slice. * $P < 0.05$.

Figure 3. (a) Safranin O-stained specimens (slice 2)

Red points denote the medial tibial eminence apex. Bars, 5 mm. (b-d) Safranin O-stained sequential section of panel a (b, anterior area; c, central area; d, posterior area). Bars, 100 μm. (e) Relative safranin O-density of the MM posterior insertion in each area (slice 2). * $P < 0.05$. (f) The cells number of each area in slice 2. * $P < 0.05$.

Figure 4. (a-c) Type II collagen deposition in the MM posterior insertion on slice 2.

Inlet in (c) showed no signal in the absence of primary antibody. Bars, 100 μm. (d) Relative type II

collagen density of the MM posterior insertion in each area (slice 2). * $P < 0.05$.