

HISTOLOGICAL AND HISTOCHEMISTRY COMPARISONS BETWEEN MEDIAL AND LATERAL MENISCI IN OSTEOARTHRITIS PATIENTS

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Abstract

Osteoarthritis (OA) is a joint disease affecting articular cartilage, menisci, and other joint components. The main objective of this study was to investigate structural and biochemical changes in the medial and lateral menisci of Thai OA patients. Histologically, the bone marrow-like structure was mainly observed at the posterior horn of both medial and lateral menisci. The numbers of meniscal cells were significantly lower at the posterior and anterior horns of the medial and lateral menisci, respectively. Cells at the posterior horn of the medial meniscus were significantly hypertrophied. A decrease in proteoglycan was observed mainly at the posterior horns of both medial and lateral menisci. In contrast, collagen fibers markedly increased at the posterior horn of the medial meniscus. Moreover, apoptotic cells were significantly higher in numbers at the posterior and anterior horns of the lateral and medial menisci respectively. The results revealed that the pathology of OA occurred mostly at the posterior horn of the medial meniscus.

Keywords: Apoptosis; Cartilage; Meniscus; Osteoarthritis; Pathology

Introduction

Osteoarthritis (OA) is a public health issue. The knee joint is one of the most affected joints by OA (Badlani *et al.*, 2013). Cui *et al.* (2020) reported that the pooled global frequency of knee OA was 16.0% in people aged 15 and above and 22.9% in people aged 40 and above. A survey study among Thai elderly people, aged more than 50 years old, revealed that the frequency of knee OA varied from

34.5% to 45.6% (Roopsawang and Aree-Ue, 2015). The factors associated with knee OA include gender, age, weight, joint trauma associated with repetitive movements in particular squatting and kneeling, and sedentary work. Three criteria symptoms utilized for the diagnosis of knee OA are persistent knee pain, limited morning stiffness, and reduced function of the knee. Other signs including

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crepitus, restriction of joint movement and bony enlargement are also useful for knee OA diagnosis.

In OA, damage of the superficial layer of the articular cartilage allows the cartilage of the connecting bone to rub together. The cartilage is the tissue that vasculature and nerve innervation are absent. It is not capable of producing inflammation or pain, at least in the initial stages of OA. Thus, the cause of pain is mainly from alterations to the non-cartilaginous tissues of the joint, for example, the articular capsule, synovial membrane, subchondral bone, ligaments, and muscles around joints (Mora *et al.*, 2018). Meniscus is fibrocartilaginous tissue which inserts between the condyles of the femur and the tibial plateau (Messner and Gao, 1998). The meniscus is generally comprised of water, collagens, and proteoglycans (Fox *et al.*, 2012). There are several collagen types in the meniscus, including types I, II, III, V, and VI. However, type I collagen is the most abundant compared to other types (McDevitt and Webber, 1990; López-Franco and Gómez-Barrena, 2018). The meniscus plays multiple physiological roles including load distribution, joint stabilization, lubrication, and sensory responsibilities (Milachowski *et al.*, 1998). Meniscus injuries can occur because of degenerative changes in the knee joint. Knee OA can begin with meniscus deterioration involving degenerative lesions and subsequent progression to knee OA due to the disappearance of meniscus function (Englund *et al.*, 2009; Hayashi *et al.*, 2023).

Apoptosis is a controlled cell death process that is complicated in the progress of homeostasis and aging. Apoptosis induces pathologies, for example, cancer, diseases, and disorders of development (Elmore, 2007). In OA, the cartilage becomes hypocellular and often appears by lacunar flushing. It is confirmed that chondrocyte mortality is related to apoptosis (Bhatia *et al.*, 2014). Histological studies which revealed decreasing in cell intenseness and lacunar emptying within OA cartilage suggest that death of cells could arise throughout the OA processes and is likely associated with OA onset (Adams and Horton, 1998; Blanco *et al.*, 1998; Hashimoto *et al.*, 1998; Héraud *et al.*, 2000; Millucci *et al.*, 2015).

Currently, effective pharmaceutical therapy for OA is still limited due to the complicated pathology of knee OA. Better understanding of the pathology of knee OA are thus needed for treatments and preventions. Various studies have focused on the meniscus relating to the origination/advancement of knee OA (Ozeki *et al.*, 2022). Therefore, the main objective of the present study was to investigate the structural and

biochemical changes of the menisci during the initial stage of knee OA. Comparisons of the changes at the medial and lateral menisci were also performed.

Materials and Methods

Tissue collection

Menisci were collected from patients who underwent total knee replacements at the Suranaree University of Technology Hospital with patients' consent and confidentiality. The procedures were granted by the Human Research Ethics Committee, Suranaree University of Technology (EC-60-53). Grades of OA were classified using the criteria reported by Kwok *et al.* (2016). Menisci from grade II knee OA patients were defined as the early stage of OA and were used in the experiments ($n = 20$). Menisci from grade II knee OA were used because the pathology of grade II knee OA was more obvious than in grades 0 and I. In addition, menisci of grades III and VI knee OA were difficult to obtain because of unfavourable knee replacement outcome risks associated with the increasing ages of these patients. The menisci were separated into parts: anterior horn, middle part, and posterior horn. Each part was separated into superficial, outer, and deep zones (Figure 1).

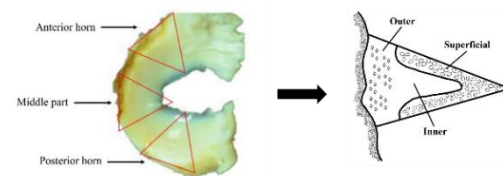


Figure 1. Parts and zones of the menisci

Tissue preparation for histological study

The menisci were fixed for 24 hours in 4% formalin solution and then dehydrated with a graded series of ethanol (70%-100%). Subsequently, the tissues were embedded in paraffin. The tissue blocks were cut serially at the thickness of 4 μm with a rotary microtome. The tissue sections were conventionally stained with hematoxylin and eosin (Bio-Optica, Milan, Italy).

Counting of meniscal cell numbers and measuring of cell sizes

The numbers and sizes of meniscal cells in various zones and horns of the medial and lateral menisci were compared. Cell sizes were measured from 200-400 cells/tissue using Cell D Imaging Software.

Proteoglycan staining

Alcian blue staining was used for histological visualization of proteoglycans. Briefly, the sections were deparaffinized and rehydrated, followed by incubation with Alcian blue, pH 2.5 (Acros Organics, Geel, Belgium) for 1 hour. Tissues were then incubated with nuclear fast red (Abcam, Boston, MA, USA) for nuclear staining.

Staining of collagen fibers

Staining of collagen fibers was performed using Picro-Sirius Red (Abcam, UK). Tissue sections were deparaffinized and hydrated in distilled water. Adequate amounts of Picro-Sirius Red were applied and incubated with the sections for 1 hour. The tissues were then rinsed quickly in acetic acid, dehydrated, and cleared with xylene.

Detection of cell apoptosis by TUNEL assay

Tissue sections were deparaffinized, submerged in 95% and 70% ethanol, and then PBS. Proteinase K (Gibco BRL, Gaithersburg, MD, USA) was applied to the sections. Endogenous peroxidase was eliminated with hydrogen peroxide (3%) (Sigma Aldrich, St. Louis, MO, USA). An equilibration buffer was added and incubated. The sections were then applied with working Strength TdT Enzyme and incubated in a humidified chamber at 37°C for 1 hour, followed by adding of stop/wash buffer.

Subsequently, the sections were incubated in a humidified chamber for 30 minutes at room temperature with anti-digoxigenin conjugate. The color was developed with a peroxidase substrate. The sections were counterstained with methyl green (0.5% (w:v)). After that, the sections were washed in 3 changes of 100% n-butanol and finally dehydrated.

Statistical analysis

The numbers and sizes of cells, and the numbers of apoptotic cells (expressed as mean $\pm$ S.D.) compared between medial and lateral menisci were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. The probability values less than 0.05 ($P < 0.05$) were considered to be significantly different.

Results

Gross anatomy of menisci in knee OA

In knee OA, structural change was observed at both articular cartilage and menisci. Abrasions were found on the articular cartilage (Figure 2(a)) and

tears were observed at the medial and lateral menisci, especially at the border of the menisci (Figure 2(b)).

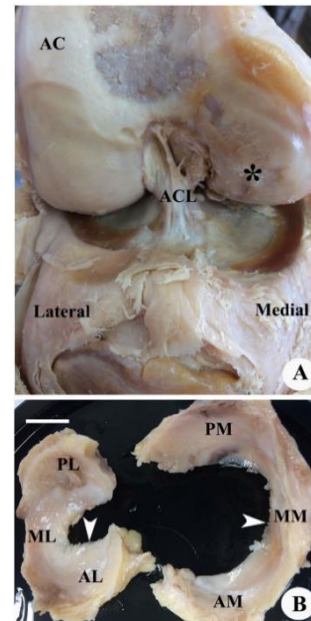


Figure 2. Gross anatomy of the knees. (a) Knee OA showing an abrasion on the articular cartilage (asterisk). (b) Knee OA showing abrasions and tears on the border of menisci (white arrowheads). AC, articular cartilage; ACL, anterior cruciate ligament; AL, Anterior horn of lateral meniscus; AM, Anterior horn of medial meniscus; ML, Middle part of lateral meniscus; MM, Middle part of medial meniscus; PL, posterior horn of lateral meniscus; PM, Posterior horn of the medial meniscus. Scale bar, 1 cm.

Histology of menisci in knee OA

Menisci of knee OA showed notable histological changes. Large areas of bone marrow-like structure were observed at the posterior horn (Figure 3(A), (B) and (D)) and middle part (Figure 3(G) and (K)), especially at the outer and deep zones, of the medial and lateral menisci. The bone marrow-like structure was rarely found at the anterior horn of the medial and lateral menisci (Figure 3, (M-R)). The superficial zone of all horns of both medial and lateral menisci showed no bone marrow-like structure (Fig. 3(C), (F), (I), (L), (O), and (R)). Large abrasions were identified mainly at the anterior horn of both medial and lateral menisci. Moreover, areas of hypercellularity was observed at the superficial zone of the middle part of the medial menisci (Figure 3(I)).

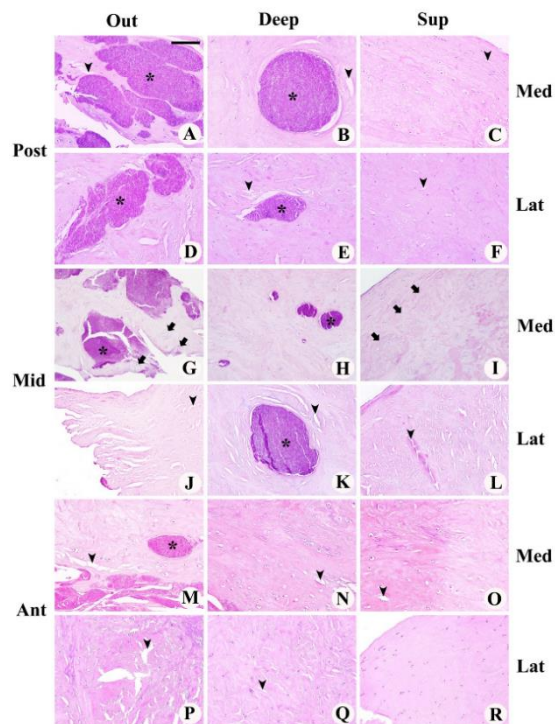


Figure 3. Menisci of knee OA showing bone marrow-like structure (asterisks), abrasions (arrowheads), and hypercellularity (arrows). (A-F) Posterior horn (Post); (G-L) Middle part (Mid); (M-R) Anterior horn (Ant). Deep, deep zone; Lat, lateral meniscus; Med, medial meniscus; Out, outer zone; Sup, superficial zone. Scale bar, 200 μ m

Numbers of cells in menisci of knee OA

Comparison of numbers of meniscal cells between medial and lateral menisci is shown in Figure 4. All zones of the posterior horn of the medial menisci showed significantly lower numbers ($p < 0.05$) of the cells (outer zone, 20.8 ± 2.7 ; deep zone, 19.4 ± 3.8 ; and superficial zone, 49.4 ± 5.5 cells) compared to the corresponding zones of the lateral menisci (outer zone, 65.8 ± 3.0 ; deep zone, 46.4 ± 3.9 ; and superficial zone, 69.8 ± 3.9 cells) (Figure 4(a)). In contrast, all zones of the anterior horn of the medial menisci showed significantly higher numbers ($p < 0.05$) of the cells (outer zone, 85.8 ± 7.0 ; deep zone, 47.0 ± 7.4 ; and superficial zone, 87.0 ± 3.5 cells) compared to the corresponding zones of the lateral menisci (outer zone, 19.8 ± 1.9 ; deep zone, 19.2 ± 1.9 ; and superficial zone, 27.4 ± 8.0 cells) (Figure 4(c)). However, the numbers of the cells at the outer and superficial zones of the middle part of the medial menisci were significantly higher ($p < 0.05$) than those of the lateral menisci (Figure 4(b)). This result revealed that hypocellularity occurred more at the

posterior and anterior horns of the medial and lateral menisci, respectively. Sizes of cells in menisci of knee OA.

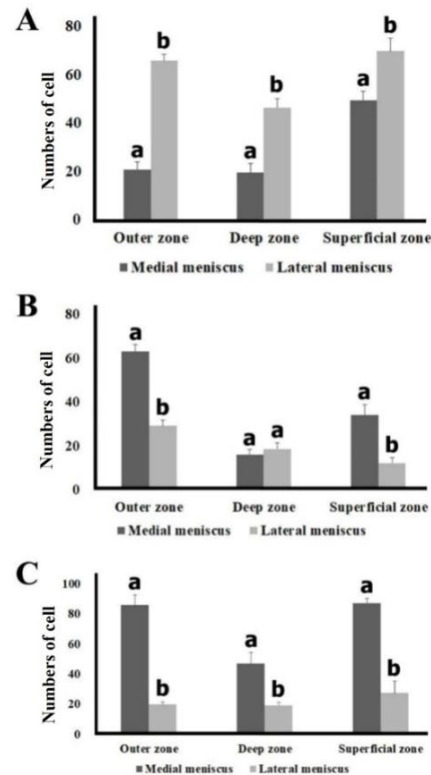


Figure 4. Numbers of cells comparison between medial and lateral menisci. (a) Posterior horn. (b) Middle part. (c) Anterior horn. The statistical difference is indicated by letters a and b.

The comparison of meniscal cell sizes between the medial and lateral menisci is shown in Figure 5. All zones of the posterior horn of the medial menisci showed significantly larger cell sizes ($p < 0.05$) (outer zone, 16.3 ± 1.7 ; deep zone, 18.6 ± 1.0 ; and superficial zone, 9.4 ± 1.2 μ m) than the corresponding zones of the lateral menisci (outer zone, 3.9 ± 0.3 ; deep zone, 3.7 ± 0.2 ; and superficial zone, 3.6 ± 0.1 μ m) (Figure 5(a)). At the middle part, the significant larger cells ($p < 0.05$) were observed only at the outer zone the medial menisci (16.5 ± 1.1 μ m) compared to those of the lateral menisci (9.2 ± 0.6 μ m) (Figure 5(b)). Moreover, at the anterior horn, significantly larger cells ($p < 0.05$) were observed only at the deep zone of the lateral menisci (5.3 ± 0.4 μ m) compared to those of the medial menisci (3.3 ± 0.4 μ m) (Figure 5(c)). This result revealed that hypertrophy of cells was observed mainly at the medial menisci, especially at the posterior horn.

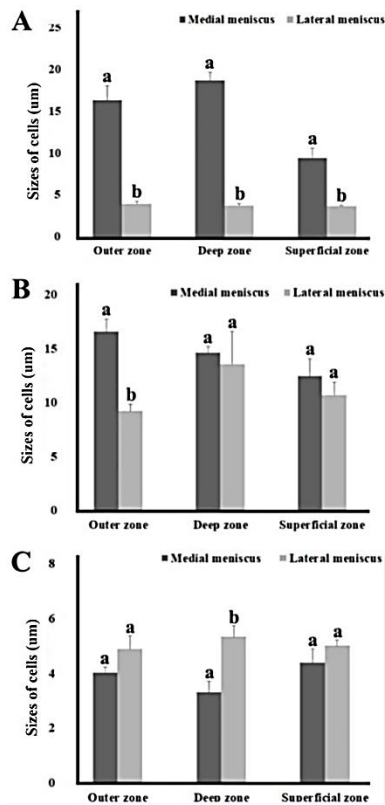


Figure 5. Sizes of cells compared between medial and lateral menisci. (a) Posterior horn. (b) Middle part. (c) Anterior horn. The statistical difference is indicated by letters a and b

Distribution of proteoglycans in menisci of knee OA

The distribution of proteoglycans compared between the medial and lateral menisci is shown in Figure 6 and summarized in Table 1. The distribution of proteoglycans was the lowest at the posterior horn of the medial (Figure 6(a-c)) and lateral menisci (Figure 6(d-e)). Other areas of both medial and lateral menisci showed more intense staining of the proteoglycans.

Table 1. Staining of proteoglycan in the menisci

Sides of menisci	Horns/Part	Zones		
		Outer	Deep	Superficial
Medial	Posterior	+/++	+/++	+
Lateral	Posterior	+	+	+
Medial	Middle	++	++	++
Lateral	Middle	++	+++	+/++
Medial	Anterior	++	++	++
Lateral	Anterior	++	++	++

Note: +, Weak staining; ++, Moderate staining; +++, Strong staining

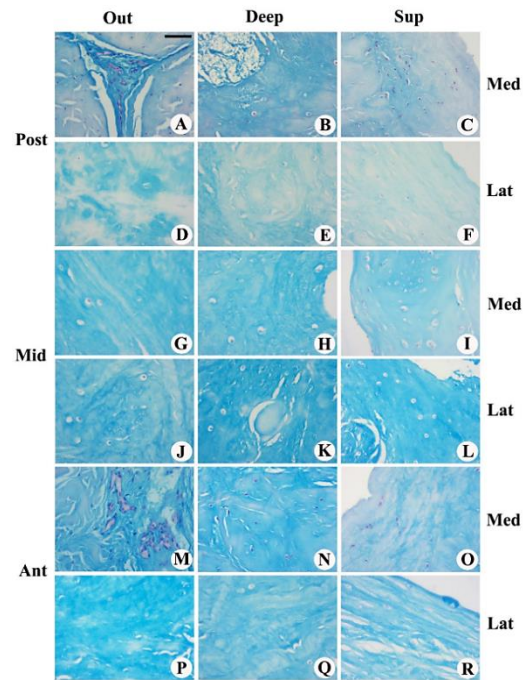


Figure 6. Proteoglycans stained with Alcian blue (pH 2.5). (a-f) Posterior horn (Post); (g-l) Middle part (Mid); (M-R) Anterior horn (Ant). Deep, deep zone; Lat, lateral meniscus; Med, medial meniscus; Out, outer zone; Sup, superficial zone. Scale bar, 200 µm

Distribution of collagen fibers in menisci of knee OA

The result of collagen fibers staining is shown in Figure 7 and summarized in Table 2. Collagen fibers were found to localize mainly at the deep (Figure 7(b)) and superficial zones (Figure 7(c)) of the posterior horn of the medial menisci. Other areas of both medial and lateral menisci showed less intensity of the collagen staining.

Table 2. Collagen fibers staining in the menisci

Sides of menisci	Horns/Part	Zones		
		Outer	Deep	Superficial
Medial	Posterior	++	+++	+++
Lateral	Posterior	++	++	++
Medial	Middle	++	++	++
Lateral	Middle	++	++	++
Medial	Anterior	++	++	++
Lateral	Anterior	++	++	++

Note: +, Weak staining; ++, Moderate staining; +++, Strong staining

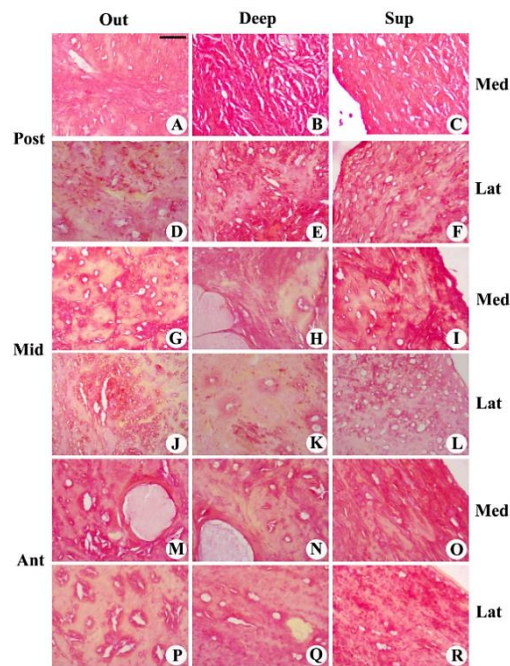


Figure 7. Collagen fibers in the menisci of knee OA. (A-F) Posterior horn (Post); (G-L) Middle part (Mid); (M-R) Anterior horn (Ant). Deep, deep zone; Lat, lateral meniscus; Med, medial meniscus; Out, outer zone; Sup, superficial zone. Scale bar, 200 μ m

Numbers of apoptotic cells in the menisci of the knee OA

The comparative quantification of apoptotic cells in the medial and lateral menisci is shown in Figure 8. At the posterior horn, apoptotic cells were significantly higher in numbers ($P < 0.05$) in the lateral menisci (outer zone, 47.7 ± 1.6 ; deep zone, 18.6 ± 1.4 ; and superficial zone, 35.2 ± 1.5 cells) than in the medial menisci (outer zone, 9.0 ± 1.0 ; deep zone, 9.0 ± 1.2 ; and superficial zone, 18.6 ± 1.0 cells). In the middle part, apoptotic cells were significantly higher ($P < 0.05$) at the superficial zone of the medial menisci (44 ± 1.1 cells) than that of the lateral menisci (20.0 ± 1.3 cells). In contrast, at the anterior horn, apoptotic cells were significantly higher in numbers ($p < 0.05$) in the medial menisci (outer zone, 28.9 ± 1.5 ; deep zone, 18.4 ± 1.2 ; and superficial zone, 29.2 ± 1.5 cells) than in the lateral menisci (outer zone, 10.2 ± 1.2 ; deep zone, 10.9 ± 1.3 ; and superficial zone, 9.5 ± 1.5 cells).

Discussion

Degenerative characteristics of the menisci are shown in knee OA. In the present study, abrasions and tears were discovered on the border of the

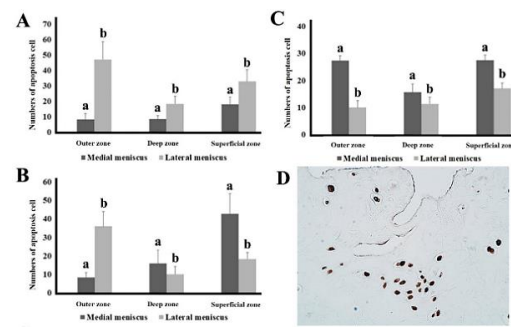


Figure 8. Numbers of apoptotic cells compared between medial and lateral menisci. (A) Posterior horn. (B) Middle part. (C) Anterior horn. (D) Apoptotic cells were detected using the TUNEL assay. The apoptotic cells stained brown in color. Statistical difference is indicated by letters a and b.

menisci. Histologically, bone marrow-like structure was found mainly at the posterior horn of the menisci. The bone marrow-like structure represented the structural change of the menisci. Normally, menisci function in supporting the knee and they relate to the distribution of forces. The structural change of the menisci could be the cause of their malfunction (Ozeki *et al.*, 2022).

Our investigation revealed that the pathogenesis of OA resulted in reduced cell numbers. Many studies reported that there was a very low proliferation of chondrocytes in OA (Meachim and Collins, 1962; Rothwell and Bentley, 1973; Hulth *et al.*, 1972; Sandell and Aigner, 2001). It is believed that the elderliness of cells may play a significant role in the pathology of OA. The senescence of chondrocytes is anticipated to give rise to the abnormal inflammatory environment present in OA (McCulloch *et al.*, 2017). Moreover, hypertrophy of cells was also observed. In the initial stage of OA, chondrocytes became larger and were clustered into clusters of cells, which are called chondrocyte clones. This was associated with the activity of cartilage and the recleaving ability of chondrocytes in OA (Bhatia *et al.*, 2014).

OA is characterized by damage to the extracellular matrix (Héraud *et al.*, 2000). Proteoglycans play a crucial role in enhancing the meniscus's ability to withstand compression forces transmitted through body movement (Mahmood *et al.*, 2020). Our investigation found that proteoglycan levels were significantly reduced, particularly at the posterior horn of the medial and lateral menisci. Additionally, a study on proteoglycan content in the canine meniscus reported a substantial decrease in proteoglycan levels at 48 hours post-injury (Nishida *et al.*, 2005).

A decline in the extracellular matrix, especially proteoglycan, affected the structure and role of the meniscus and caused the pathology of OA (Lopez-Franco *et al.*, 2018). It was proposed that the first stage of the progression of OA included the degradation of proteoglycan (Wu *et al.*, 2019). Sandell and Aigner, (2001) stated that in OA, degeneration of cartilage was specified by two phases: biosynthetic and degradative phases. During the biosynthetic phase, the cartilage cells attempted to restore the damaged extracellular matrix while during the degradative phase, the enzymes synthesized by the chondrocytes digest the extracellular matrix and the consequent erosion of the cartilage is increased. Chondrocytes are known to yield inducible nitric oxide synthase (iNOS) which response to a well-known proinflammatory cytokine in OA, interleukin 1 β (IL1 β). The iNOS causes the production of nitric oxide which reduces the formation of proteoglycan (Héraud *et al.*, 2000). Degradation of proteoglycan in OA induced severe changes in cartilage homeostasis (Bertrand and Held, 2017).

The immunohistochemical analysis revealed intense staining of collagen fibers predominantly in the posterior horn of the medial menisci. This observation aligns with previous research indicating an increased number of collagen fibers in patients with osteoarthritis (OA). Katsuragawa *et al.* (2010) reported a dramatic elevation in the expression of type I, II, and III procollagens in menisci affected by OA. Similarly, Miosge *et al.* (2004) observed an increase in collagen type I mRNA levels correlating with OA progression. In addition, studies on mice demonstrated the presence of collagen types I and II in the vascular regions of both the anterior and posterior menisci, suggesting an increase in fibrosis due to the elevated load or tension in these areas as a response to OA (Yoshioka *et al.*, 2022). Late-stage OA is typically marked by severe cartilage degradation accompanied by heightened expression of collagen type I, which is thought to facilitate mineralization of the articular cartilage (Miosge *et al.*, 2004; Hui Mingalone *et al.*, 2018). The increased collagen content in the meniscus during OA progression reflects a complex interplay of mechanisms aimed at healing, compensating for tissue degradation, adapting to heightened mechanical stress, and responding to the inflammatory environment. These findings underscore the meniscus's efforts to preserve its structural and functional integrity despite ongoing degenerative changes induced by OA (Yunus *et al.*, 2020; Terkawi *et al.*, 2022).

Cellular changes in terms of numbers and sizes were observed mostly at the medial menisci. This occurred in concert with the reduction of

proteoglycans and increased collagen fibers. It was reported that the medial meniscus was usually damaged more than the lateral meniscus (Englund *et al.*, 2008, Katsuragawa *et al.*, 2010). This could be because the medial meniscus which lies on the inner side of the knee joint supports more of the body's weight than the lateral meniscus. Because the weight is directed downward to the medial side of the knee rather than the lateral side, pathology of the medial meniscus can lead to the formation of knee OA caused by the unbalance of the body weight transferred (Badlani *et al.*, 2013; Katz and Losina, 2013).

There was additional research stating that the development of knee OA affected the posterior horn of the menisci more than the anterior horn (Badlani *et al.*, 2013). This corresponds with the results of the present study. A greater anterior to posterior horn ratio of the medial meniscus results in its less movement compared to the lateral meniscus (Fox *et al.*, 2012). Thompson *et al.* (1991) reported that motion of meniscus occurred the least at the posterior medial corner, where the meniscus was constrained by its attachment to the tibial plateau by the posterior oblique ligament of the knee. A decrease in the movement of the posterior horn of the medial meniscus was a potential mechanism for meniscal tears (Fox *et al.*, 2012). Knee biomechanism is known to be affected by posterior tibial slope and meniscal slope. As the posterior horn of the meniscus is thicker than the anterior horn, the meniscal slope is nearly vertical to the anatomical axis of the tibia and could counteract the posterior inclination of the posterior tibial slope. The meniscal slope increases, and the anterior-posterior transmission of the femoral condyle might become greater. Thus, the posterior horn of medial meniscus is more prone to injury in knees with larger medial meniscal slope (Wang *et al.*, 2020). Similarly, Moon *et al.* (2020) discovered that an increased posterior tibial slope was strongly associated with an increased incidence of tears of the posterior horn of the medial meniscus. Therefore, it was suggested that the posterior horn of the medial meniscus was an significant structure for monitoring the progression toward the treatment of knee OA (Krych *et al.*, 2018).

Pathological effects of OA can cause cellular damage leading to necrosis and/or apoptosis of cells (Salucci *et al.*, 2022). Apoptosis is an important basic biological phenomenon which is an essential process of the maintenance of cellular homeostasis and survival of multicellular organisms (Bhatia *et al.*, 2014; Héraud *et al.*, 2000). It is known that the pathology of the meniscus is related with the apoptosis of cells. The percentage of apoptotic cells was associated with the serious pathology of OA

(Hashimoto *et al.*, 1998; Lopez-Franco *et al.*, 2018). Apoptosis occurred in OA cartilage more than in normal cartilage (Héraud *et al.*, 2000). It is still unclear whether chondrocyte apoptosis is the persuader of cartilage degeneration or whether apoptosis occurs as the byproduct of cartilage destruction (Bhatia *et al.*, 2014). However, many studies suggested that cell apoptosis could be used as a benchmark for the detection of the onset or progression of knee OA (Blanco *et al.*, 1998; Bhatia *et al.*, 2014; Kim *et al.*, 2000; Héraud *et al.*, 2000). In the present study, the highest number of apoptotic cells was observed in the posterior horn of the lateral meniscus. This could be because of load-bearing capacity of the lateral meniscus. During full extension of the knee joint, the lateral meniscus transmits 70% of the load across the lateral side of the joint, whereas the medial meniscus is responsible for 50% of the load transmission through the medial side. Moreover, during 90 degrees of flexion, the lateral meniscus transmits 85% of the load (Westermann and Wolf, 2018). Total meniscectomy of the lateral meniscus results in decreased contact area (40-50%) and increased contact stress (200-300%) in the lateral knee compared to normal knee (Baratz *et al.*, 1986, Fox *et al.*, 2012). This may contribute to accelerated death of the cells. The higher number of apoptotic cells, thus, occurred the most in the lateral meniscus.

The present study provides information on the basic knowledge of meniscus in OA patients. Understanding of the histological and histochemical changes of the meniscus is essential for the effective management of OA. A viable substitute for the current standard repairment of meniscal injuries is meniscal tissue engineering. Up to date, 3D bioprinting provides alternatives to current surgical methods for meniscus repair. Although bioprinting techniques are employed in developing engineered meniscus grafts. However, 3D bioprinting must mimic the gradient structure, composition, and viscoelastic properties of native meniscus (Lv *et al.*, 2023; Chae *et al.*, 2021). Therefore, the knowledge gained from the present study could be applied to the design of a 3D-printed meniscus that increases the necessary constituents in the posterior horn of the medial lemniscus to decrease the damage (Barceló *et al.*, 2023a; Barceló *et al.*, 2023b). Moreover, in order to avoid operative management, mesenchymal stem cells have been injected to repair damage of the menisci (Chirichella *et al.*, 2019). Knowledge gained from the present study could provide insight that which location of the knee is the preferable site for stem cells injection.

In summary, our investigation discovered that reduced cell numbers, cell hypertrophy, decreased proteoglycans, and increased collagen fibers were

observed mainly at the posterior horn of the medial menisci. However, cells that underwent apoptosis were found mainly at the posterior horn of the lateral menisci. These results revealed that the part of the menisci mostly affected by the pathology of knee OA was the posterior horn, especially that of the medial menisci.

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