Dimorphic comparative histological and histometric study of the lateral and medial knee menisci in male and female human cadavers

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SUMMARY

The knee menisci are fibrocartilaginous discs present in between the femur and the tibia. They play a pivotal role in withstanding the weight-bearing forces and help to maintain the stability of the knee joint. Descriptive knowledge of the menisci is essential to understand the mechanism and design of the appropriate management of their pathological lesions. Several investigations have identified the anatomical and morphological differences between the medial (MMi) and lateral menisci (LMi) in humans. What remains unclear, however, is the comparative analysis of histological and histometric parameters of these menisci, especially between males and females. To evaluate and compare the different histological, histometric and biochemical parameters of different structural components (collagen, proteoglycans, cellularity, and vascularity) of MMi and LMi in male and female human cadavers, twenty-four knee joints, 12 males and 12 females, were dissected, and the menisci were excised and labeled. The middle region of each meniscus was cross-sectioned and subjected to different histological, histometric and biochemical studies.

Histological examination of MMi and LMi in both males and females yielded several observations regarding different meniscal components; the orientation of collagen fibers was circumferential with intermingled radial fibers. Both fibroblasts and fibrochondrocytes were arranged singly or in rows alongside the direction of the collagen fibers. Also, the blood vessels were present toward the periphery, whereas the proteoglycans-rich areas were more evident in the inner region of each meniscus. Nevertheless, collagen fibers organization, safranin-O staining intensity, and cellular arrangement were all different between males and females. The analysis of these changes was further compared histometrically between MMI and LMi in males and females. The gained comparative histological, histometric and biochemical findings of this work are critical for providing detailed information on the microstructure and composition of both MMi and LMi in males and females. The present results also highlight the detrimental

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effects of histological structure on different meniscal injuries.

Key words: Meniscus – Collagen – Proteoglycan – Cellularity – Male – Female

INTRODUCTION

The management of meniscal injuries, which impair the proper function of the knee, remains difficult and challenging. Although several clinical options are available for the treatment of such injuries, complete cure of the damaged meniscus has proved difficult due to the limited healing capacity of the tissue (Ozeki et al., 2021).

The complex and multiple functions of menisci depend on their unique cellular and biochemical composition, perhaps more importantly on the organization and interactions of their constituents. In general, the basic structure of menisci is not homogeneous, and their composition differs with age, in injury or under pathological conditions (López-Franco and Gómez-Barrena, 2018; Battistelli et al., 2019).

Histologically, two types of cell populations have been identified in the meniscal cartilage; the fusiform cells, similar to the fibroblasts, located along the superficial zone of the meniscus, and the other polygonal cells, the fibrochondrocytes, located interior to the superficial margin. The collagen fibers, predominantly type I, form a network that entraps different types of cells in proteoglycans-rich extracellular matrix (McDevitt and Webber, 1990; Fox et al., 2015; Puetzer and Bonassar, 2016). It was also reported that the orientation of collagen fibers changes throughout the cross-sectional surface of the meniscus depending on the function of each region. Based on that, the meniscal structure consists of three distinct layers: a superficial layer, a lamellar layer, and a deep layer. The superficial layer is composed of randomly oriented fibers that provide a smooth and lubricated surface for articulation. The lamellar layer, immediately deep to the superficial layer, is also composed of randomly oriented fibers and interspersed with radially oriented fibers. While in the deep layer of the cartilage, fibers are arranged circumferentially and bound together by interspersed, radially oriented tie fibers to provide the structural support. This organization allows the conversion of vertically oriented compressive forces into circumferential hoop stresses distributed throughout the meniscus (Rattner et al., 2011; Andrews et al., 2014). On the other hand, two distinct regions of different collagen fibers exist; the inner one-third bundles having a radial pattern, and the outer two-thirds fibers are oriented in a circumferential manner (Cengiz et al., 2017; Danso et al., 2017).

Biochemically, the normal meniscal tissue is highly hydrated and predominantly composed of water (65-75%), while the remaining 20-25% consists of organic component, namely extracellular matrix proteins (ECM) and cells. The ECM that surrounds meniscus cells is largely composed of several types of collagens (75%), proteoglycans (GAGs) (17%) and small percentages of adhesion glycoproteins. Meniscus composition differs with age, in injury or under pathological conditions (Makris, 2011; Cengiz et al., 2017; Bahcecioglu et al., 2019).

Reviewing the literature, we found that there was some available data, which support that the women are more affected and burdened by knee osteoarthritis (OA) than men. Nevertheless, the etiology and implications for this sex difference remained unclear (Zhou et al., 2018; Pringle et al., 2019). In our previous study (Aggad et al., 2022), we found several differences of morphological and morphometric parameters of the MMi and LMi between males and females, which supported this gender-specific differences. Also, it was assumed that the alteration in cartilage composition, acting together with different walking mechanics between men and women, can potentially predispose women to a greater risk of OA (Blagojevic et al., 2010; Wise et al., 2012; Litwic et al., 2013; Kumar et al., 2015; Battistelli et al., 2019; Peshkova et al., 2022).

Also, it was documeted that the function of meniscal fibrocartilage is dependent mainly on the structural and biochemical components of ECM; during degenerative conditions, the changes of collagen and proteoglycan contents could impair cartilage integrity (Alaqeel et al., 2020). Therefore, we aimed in this work to evaluate and compare the

different histological, histometric, and biochemical parameters of the structural components (collagen, proteoglycans, cellularity, and vascularity) of MMi and LMi in both male and female human cadavers. Further insight into the basic science of meniscal structure is necessary for the diagnosis of different knee injuries and for guiding the treatment strategies for such diseases.

MATERIAL AND METHODS

Cadaveric material collection

Twenty-four human embalmed cadavers (12 males and 12 females), aged between 50 and 70 years, were chosen from the dissection lab of the department of Anatomy, faculty of medicine at King Abdulaziz University (KAU). The study was done under the approval of the Biomedical Ethics Research Committee unit at the Faculty of Medicine, KAU (HA-02-J-008). The normality of the cadavers' knee-joint regions was confirmed macroscopically at the time of sample collection. After removing the skin and muscles, the knee joints were dissected anteriorly by a longitudinal incision on each side of the knee-joint capsule, and by cutting the patellar ligament and the collateral ligaments transversely. To expose the menisci clearly, the joint capsule and the intra-articular ligaments (anterior and posterior cruciate ligaments) were cut to reveal the tibial plateau. The menisci in pairs of right (Rt) and left (Lt) sides, and from male and female cadavers, were excised gently by cutting their anterior and posterior attachments from the tibial plateau with scalpel blades. The menisci were then numbered, labeled, and kept in 10% buffered formalin solution for 1 week for the downstream experiments.

Histological Studies

Chemicals and reagents used in tissue processing and staining techniques were purchased from sigma-Aldrichunless otherwise stated. Tissue processing for histological techniques was performed using the protocol described recently by Charnwichai et al. (2023). Each meniscus was cut transversely at the middle point of the body to prepare 2 mm thick triangular pieces. Consequently, both femoral and tibial surfaces, as well as the inner and outer regions were exposed as shown in Figs. 1-4. The meniscal pieces were then immersed in 14% of Ethylene-diamine-tetra-acetic acid (EDTA) solution for 3 days to enhance the softening of the tissues. Following this step, the meniscal tissues were manually dehydrated in a graded series of ethanol concentration (50, 70, 80, 90, and 100%, for 20 min each), cleared with xylene for 20 min, and embedded in paraffin wax. A minimum of 6 sections, 5 μm thick each, were produced per block using LEICA RM 2255 (Leica Microsystem, Germany).

The sections were then stained by Hematoxylin and eosin (H&E), Masson Trichrome (MT) and Safranin-O (Saf-O) stains following the previously described methods by Bancroft et al. (2013);

Fig. 1.- Representative photomicrograph showing the gross appearance and the sectioning site of the MMi and LMi: sections were made across the radial width in the body middle-segment of each LM (A) and MM (B) respectively.

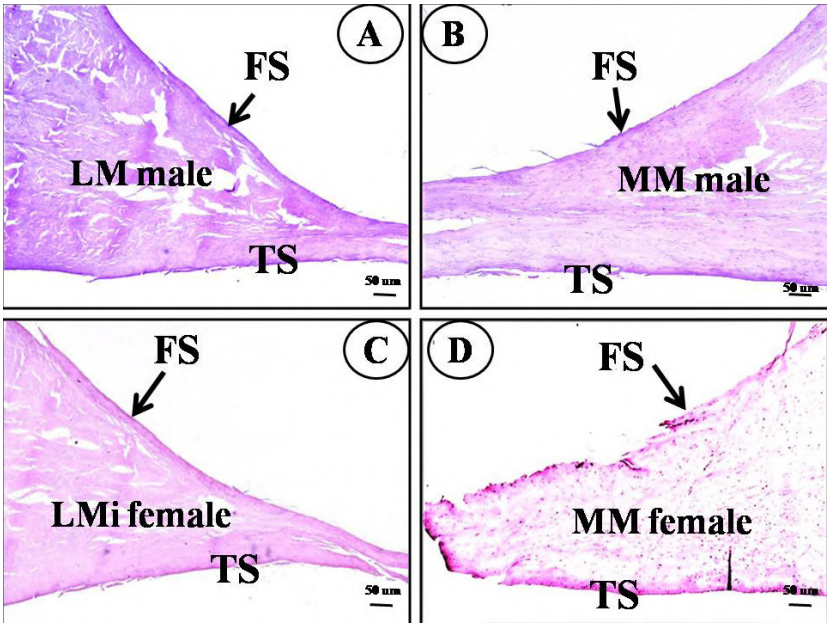

Fig. 2.- Cross sections from middle portion of LMi and MMi in male (**A,B**) and female (**C,D**) cadavers: FS = femoral surface, TS = tibial surface. H&E staining (x40). Scale bars = 50 µm.

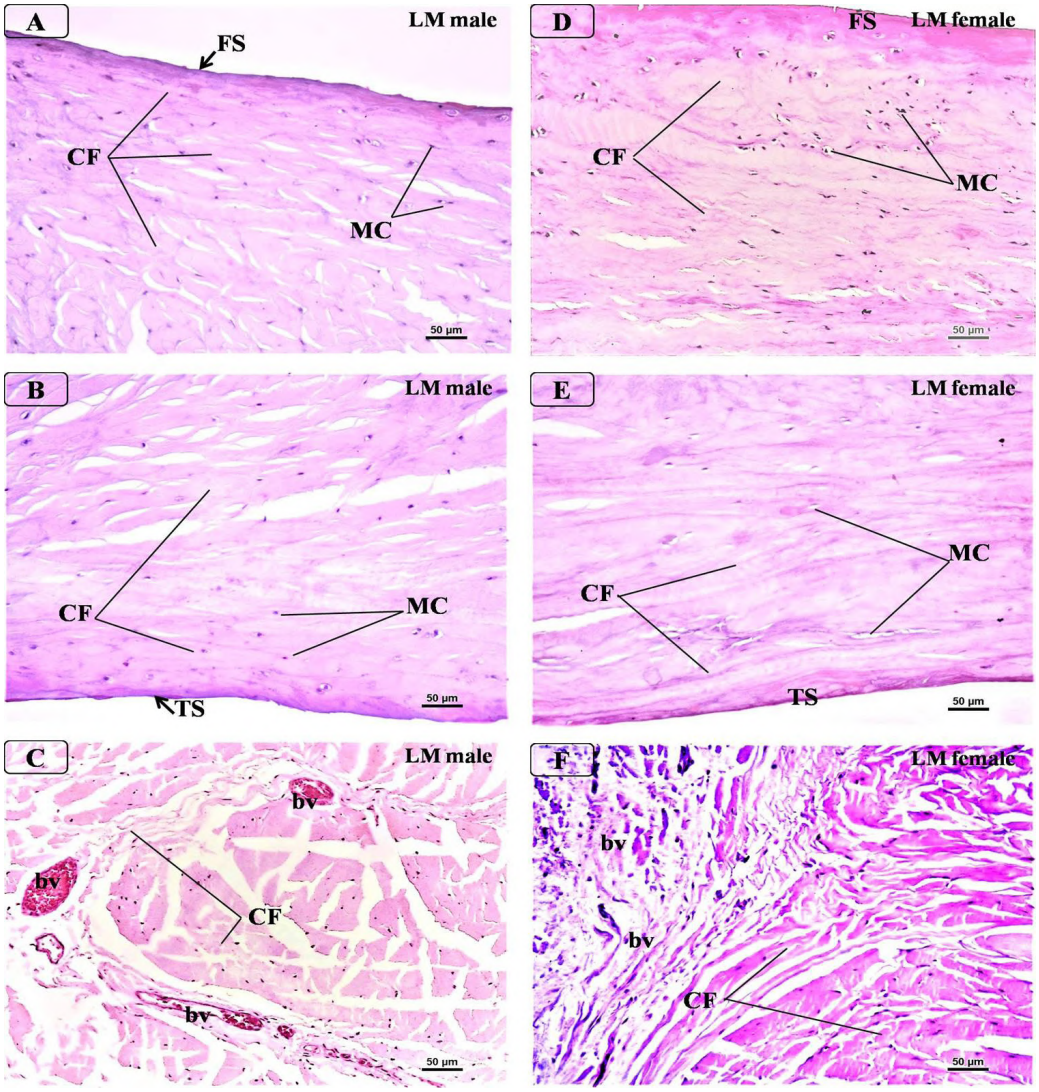

Fig. 3.- Cross sections LMi in male and female cadavers: **A**) femoral surface (FS) of male LM, **B**) tibial surface (TS) of male LM, **C**) peripheral part of male LM, **D**) femoral surface (FS) female LM, **E**) tibial surface (TS) of female LM, **F**) peripheral part of female LM. CF = collagen fibers, MC = meniscal cells, bv = blood vessels. H&E staining (x200). Scale bars = 50 µm.

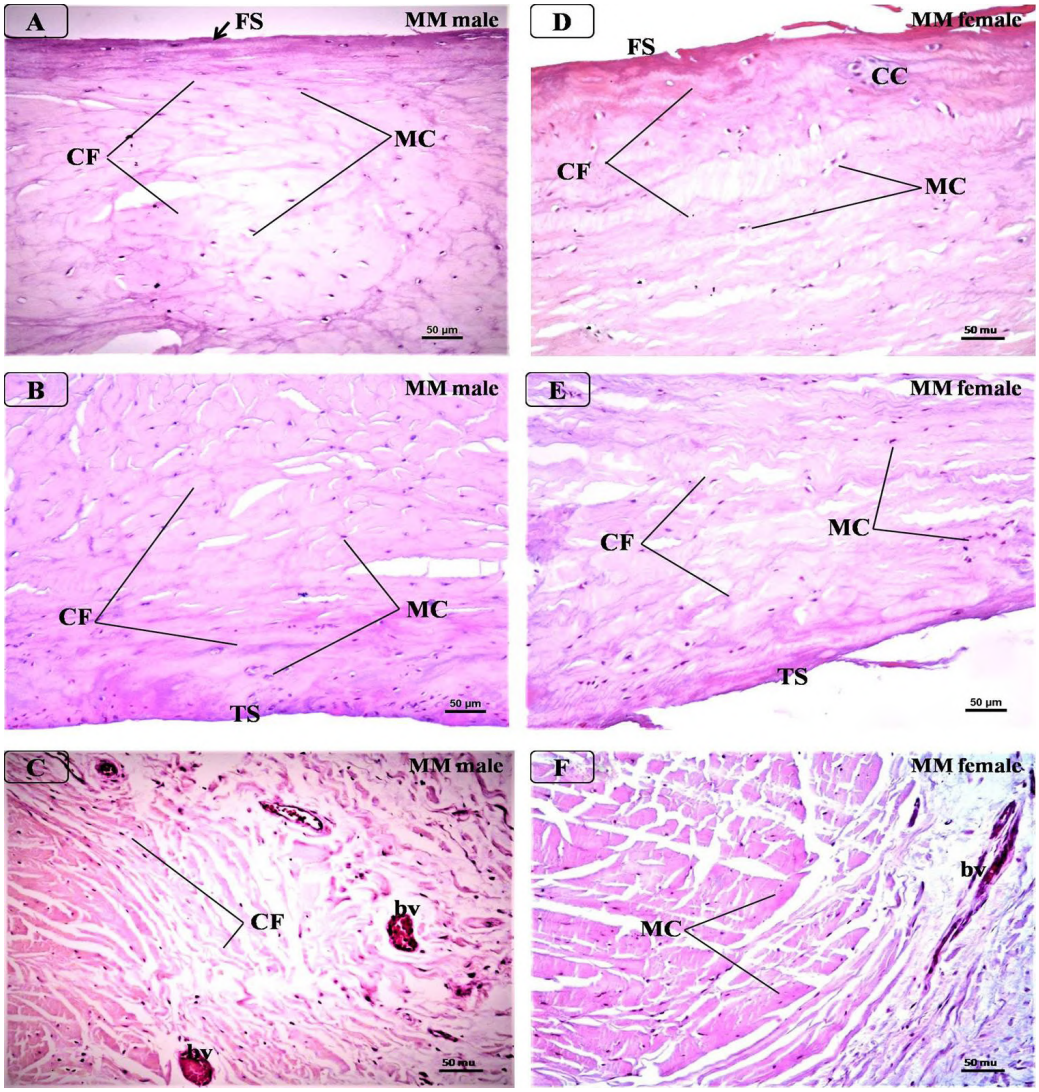

Fig. 4.- Cross sections MMi in male and female cadavers: **A**) femoral surface (FS) of male MM, **B**) tibial surface (TS) of male MM, **C**) peripheral part of male MM, **D**) femoral surface (FS) female MM, **E**) tibial surface (TS) of female LM, **F**) peripheral part of female MM. CF = collagen fibers, MC = meniscal cells, bv = blood vessels. H&E staining (x200). Scale bars = 50 µm.

H&E staining was used for depicting the general structure, cellularity and vascularity of tissues, MT stain was used to estimate the collagen fibers content and organization whereas Saf-O stain was used for assessing the content and distribution of the glycosaminoglycan (GAG) (Bancroft et al., 2013). Finally, all histological preparations were examined and representative photomicrographs, snapped under Olympus BX41 photomicroscope equipped with an Olympus DP25 digital camera (Olympus, Tokyo, Japan), were obtained.

Histometric Studies

With the use of image analysis software (Image J, version 1.45s, National Institutes of Health, Bethesda, USA), we obtained a quantitative assessment of the cellularity and vascularity of the tissues, the distribution and orientation of collagen fibers and Saf-O staining of proteoglycans. In brief, six photomicrographs were taken on each section at the magnification of X200. On each photo, the scoring system of degenerative changes adopted from Pauli et al. (2011) was used herein with some modifications to grade the morphological criteria in the tissues from (0) to (3) as illustrated in Table 1.

Biochemical Analyses

a) Determination of hydroxyproline (Hyp) content

The amount of free hydroxyproline in the meniscal extracts was quantified utilizing the method described previously by Reddy and Enwemeka (1996). The LMi and MMi pieces were homogenized

Table 1. Histometric analysis of the menisci. A modified scoring system of the degeneration changes in meniscal tissues based on histometric analysis of 4 parameters. Each parameter was given a score from 0 to 3 depending on the observational findings.

Criteria	Histological features	Score
Cellularity (H & E)	A. Normal arrangement of cells	0
	B. Disorderly arrangement of cells	1
	C. Hypercellularity	2
	D. Hypocellularity (empty lacuna)	3
Vascularity (H & E)	A. No vascularity	0
	B. Little vascularity	1
	C. Moderate vascularity	2
	D. Extensive vascularity	3
Collagen fibers (MT)	A. Well-arranged collagen fibers	0
	B. Moderately arranged collagen fibers	1
	C. Slightly arranged collagen fibers	2
	D. Distorted collagen fibers	3
GAG staining (Saf-O)	A. Minimal pink-red staining	0
	B. Slightpink-red staining	1
	C. Moderate pink-red staining	2
	D. Strong pink-red staining	3

and hydrolyzed in 2N of sodium hydroxide (Fisher Scientific, R74700501A). The samples were then incubated for 6 hours at 105°C, after which they were mixed with a buffered chloramine-T reagent (sigma, 402869, 98%). The oxidation was allowed to proceed for 25 minutes at room temperature, and the reaction started by adding 0.5M of a freshly prepared Ehrlich's reagent (Sigma, D2004), followed by incubating samples at 65°C for 20 minutes with shaking. Each sample was measured in duplicate, and the absorbance of reddish-purple complex in each sample was measured at A_{530} nm using a microplate reader (Thermo, Kyoto, Japan). The collagen value was calculated, assuming that 13.5% of collagen is Hyp, which was used as the conversion factor for calculating collagen content.

b) Determination of Proteoglycan content

The sulphated glycosaminoglycan content of meniscal tissues was quantified using the Dimethyl methylene Blue (DMB) assay previously described in Farndale et al. (1986). First, the DMB color reagent was prepared by dissolving 16 mg of DMB (Serva Fein biochemica, 931418-92-7) in 1:1 water containing 3.04 g glycine, 2.37 g NaC1 and 95 ml 0.1 M HC1, to give a solution at pH 3.0. To digest the interfering proteins or glycoproteins, LMi and MMi tissue samples were incubated with 1ml of papain solution made of (20 mM sodium phosphate buffer (pH 6.8), 1 mM EDTA, 2 mM dithiothreitol, and 300 µg papain) at 65°C for 6 hours to ensure a full digestion of all tissues. For the selective digestion of glycosaminoglycan, the papain-digested samples were incubated in chondroitin AC lyase (merk, 904757) for 30 min at 37°C, after which they were mixed with the previously prepared DMB to form the sulphated GAG-DMB complex. The colour reactivity of this complex was measured at A_{525}nm using a microplate reader (Thermo, Kyoto, Japan).

Data Analysis

Data were analyzed using IBM SPSS Statistics software for Windows, version 23 (IBM SPSS, IBM Corp., Armonk, N.Y., USA). Shapiro-Wilk test was used to evaluate normal data distribution. Statistical comparisons between male and female made by unpaired student *"t"* test and in the same group by paired student *"t"* test. Data were expressed as mean ±standard deviation (SD) and a *P*-value ≤ 0.05 was considered significant.

RESULTS

Histological findings of LMi versus MMi in male and female cadavers

The cross sections of MMi and LMi allowed studying of their fibrocartilagee from both the femoral and the tibial surfaces. Our microstructure analysis

addressed the compositional aspects of the menisci, and our approach was oriented to the meniscal cells, the extracellular matrix components, particularly collagen fibers and proteoglycans. Additionally, we explored the vascularity in various regions of the menisci. We found variations in the spatial distribution of these elements and in architectural characteristics among MMi and LMi in both sexes.

a) H & E-staining

Tables 2 and 3 presented the histological analyses of H&E-stained sections derived from male and female groups. The lateral and medial menisci were separately compared between genders; refer to Tables 2 and 3 and the related figures for a concise presentation of the key histology findings.

b) MT staining

Sections stained with MT allowed the identification of the new (blue) and old (red) collagen fibers in the meniscus. The majority of male LMi and MMi tissues depicted in Fig. 5A and B exhibited a robust MT reactivity, implying a greater presence of collagen. Nonetheless, certain MMi sections

Table 2. Comparison of histological findings in lateral menisci between male and female groups.

	Male LMi (Figure 3 A, B, C):	Female LMi (Figure 3 D, E, F):
Collagen fibers Oriantation and Vascularity	▪ The collagen fibers demonstrated an organized orientation, aligning parallel to the surfaces of both the femur and tibia. ▪ In the inner portion, the collagen fibers exhibited a circular orientation, while in the outer portion, they displayed a circumferential arrangement characterized by fewer tightly packed radial fibers and a higher density of blood vessels.	▪ Compared to the LMi in males, the collagen fibers oriented towards the femoral and tibial surfaces exhibited a heightened radial alignment. ▪ Within the inner portion, the fibers displayed a densely concentrated radial orientation, whereas in the outer segment, they showcased a circumferential arrangement featuring closely packed radial fibers and a reduced number of blood vessels.
Cellularity	▪ The predominant cellular type in the meniscus was fibroblast-like, particularly in proximity to the surfaces. In the central region, cells exhibited a polygonal shape reminiscent of fibrochondrocytes.	▪ Meniscal cells, displaying a fibroblast-like morphology, were predominantly situated near the surfaces, while a higher concentration of fibrochondrocytes was evident in the central region.

Table 3. Comparison of histological findings in medial menisci between male and female groups.

	Male MMi (Figure 4 A, B, E):	Female MMi (Figure 4 D, E, F):
Collagen fibers Oriantation and Vascularity	▪ The alignment of collagen fibers bore similarity to LMi, albeit with a less regular arrangement of superficial fibers near the surfaces. ▪ In the inner region, the fibers exhibited a network that was less tightly packed compared to LMi. Conversely, in the outer region, they demonstrated a circumferential orientation intertwined with radial fibers and a lower density of blood vessels.	▪ The collagen fibers in female MMi were not as densely packed as those in male MMi. In certain regions, the fibers appeared less compact and disorganized. ▪ Both the inner and outer portions exhibited an uneven distribution of collagen fibers, alongside fewer blood vessels intermixed within the matrix.
Cellularity	▪ The arrangement of meniscal cells resembled that of LMi; nonetheless, a greater presence of fibroblast-like cells was detected near the surface and central regions.	▪ The arrangement of meniscal cells was characterized by irregular patterns, and instances of increased cell density were noted in the form of clusters near the surfaces of the meniscus.

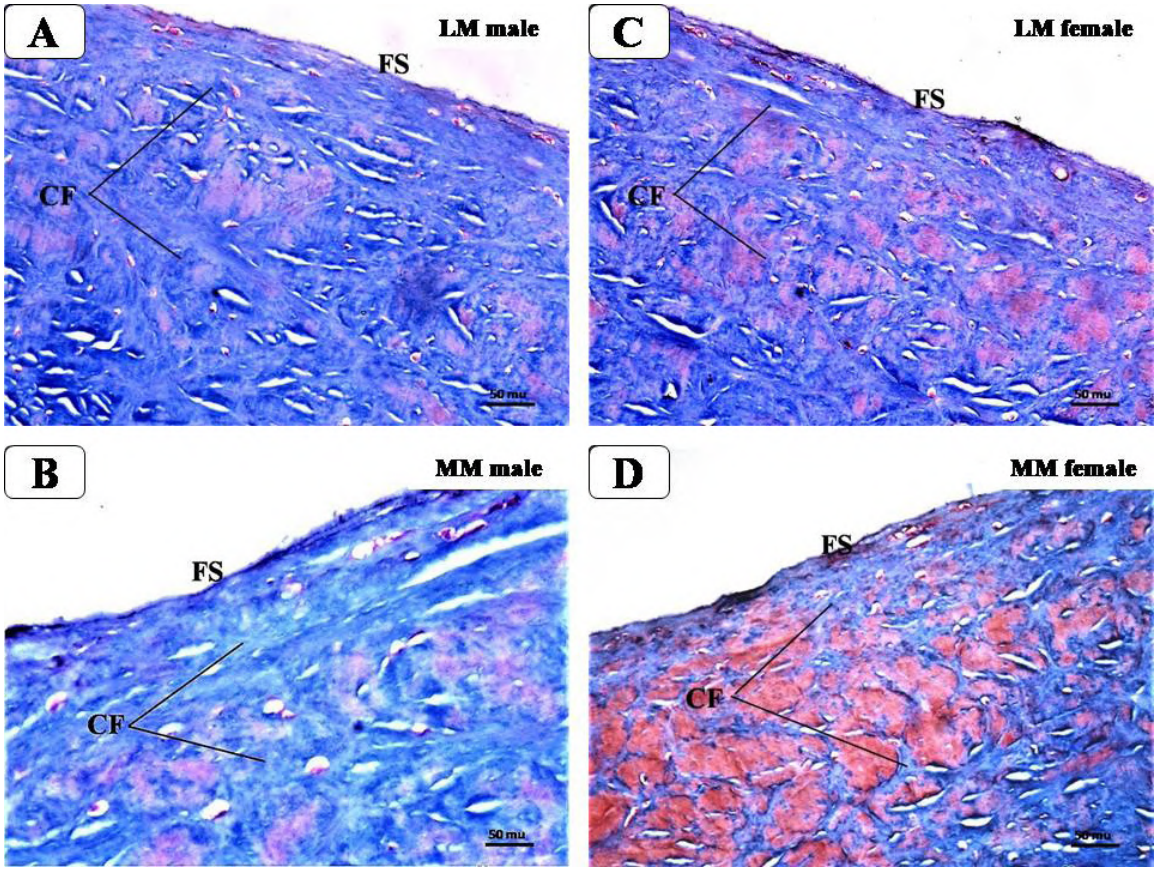

Fig. 5.- Cross sections LMi and MMi in male (**A,B**) female (**C, D**) cadavers: Masson Trichrome staining (x200). Scale bars = 50 µm.

displayed reduced levels of newly formed collagen fibers compared to LMi, evident through weak blue staining. Furthermore, the collagen fiber density on the femoral side of MMi was less than that on the tibial side. Within female menisci, sections with MT (Fig. 5C, D) exhibited comparatively weaker staining for both LMi and MMi, signifying a diminished collagen content, particularly noticeable in MMi when compared to their male counterparts. Additionally, it was evident that the density of fibers facing the femoral side was lower than those oriented toward the tibial side.

c) Saf-O staining

Saf-O statining was utilized to visualize the presence of proteoglycans (GAGs) within the meniscal tissue, indicated by a pink to red coloration. Both in males and females, a notable difference in the intensity of Saf-O staining (represented by a red hue) were between the peripheral (Fig. 6) and the central region (Fig. 7) of both LMi and MMi. Notably, both LMi and MMi showcased heightened GAG content in their central regions compared to the peripheral regions, where GAG stating was less prominent and sporadic. Upon comparing male and female menisci, a diminished stating intensity was observed in the peripheral parts of female LMi and MMi when contrasted with their male counterparts. Nevertheless, the central areas of female LMi demonstrated a more robust staining intensity when compared to male menisci.

Histometric findings of LMi versus MMi in male and female cadavers

Table 4 and Fig. 8 present the histometric findings encompassing cellularity, vascularity, collagen fiber arrangement, and Safranin-O staining for both male and female menisci. Our analysis unveiled significant alterations in microstructural integrity, evident by the cellular disorganization and increased cell populations within male MMi compared to LMi ($P = 0.01$). Conversely, the distribution of cells within the female menisci exhibited no significant change, ($P= 0.39$). Furthermore, while statistical significance wasn not reached, a more pronounced degree of cellular disorganization was observed in female LMi when compared to its male equivalent. This similar trend of cel-

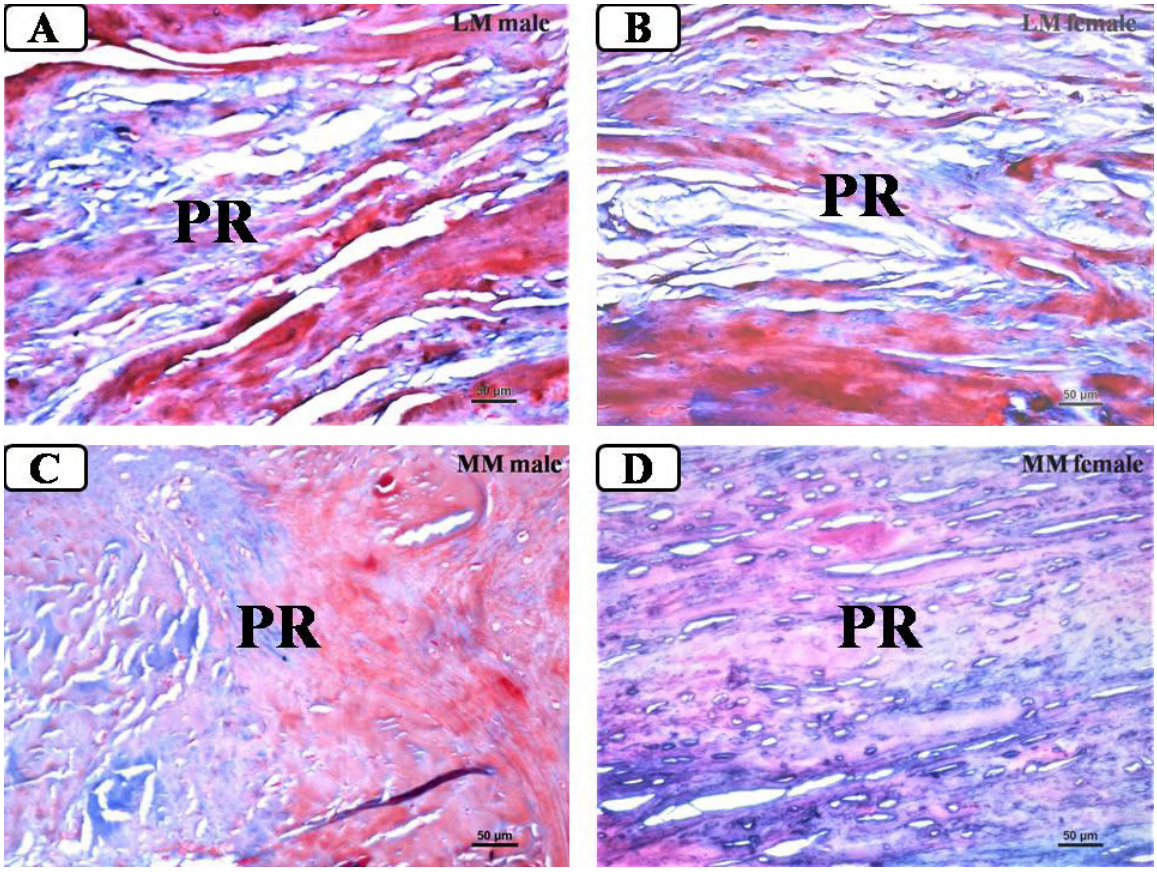

Fig. 6.- Saf-O-stained sections in the peripheral regions (PR) of male LMi (**A**) and female LMi (**B**), male MMi (**C**) and female MMi (**D**): Saf-O staining (x200). Scale bars = 50 µm.

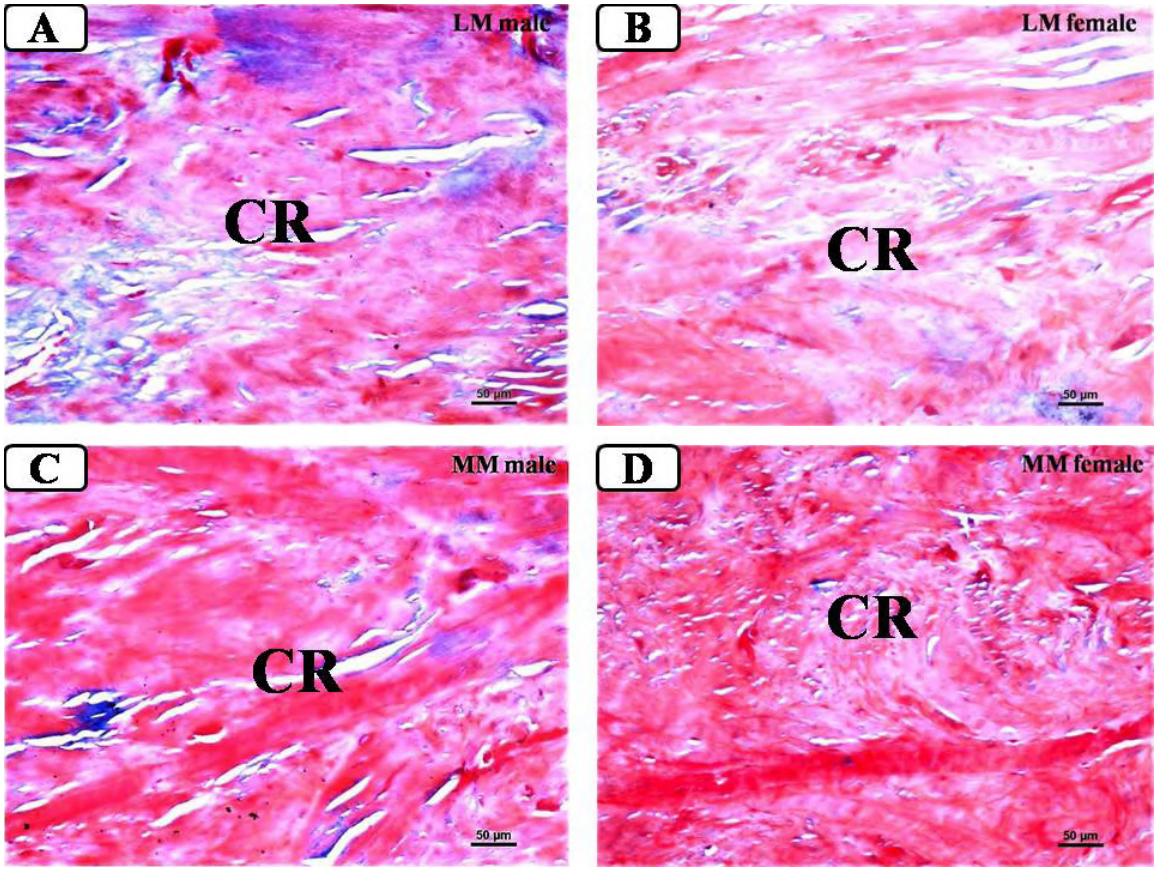

Fig. 7.- Saf-O-stained sections in the central regions (CR) of male LMi (**A**) and female LMi (**B**), male MMi (**C**) and female MMi (**D**): Saf-O staining (x200). Scale bars = 50 µm.

Table 4. Histometric findings of MM versus LM in male and female cadavers: Data were expressed as mean+/- Standard deviation. P: significance LMi versus MMi; P: significance male versus female.

Parameters	LMi (males)	MMi (males)	LMi (females)	MMi (females)
Cellularity	1.48 ± 0. 24	1.72 ± 0. 18	1.66 ± 0. 33	1.77 ± 0. 28
Significance	-	^{1}P =0.0111	^{2}P=0.1407	^{1}P=0.3881 ^{2}P=0.608
Vascularity	2.16 ± 0. 54	2.42 ± 0. 38	2.55 ± 0. 75	2.77 ± 0. 66
Significance	-	^{1}P =0.1864	^{2}P=0.1579	^{1}P=0.4537 ^{2}P=0.1257
Collagen fibers	1.23 ± 0. 15	1.54 ± 0. 17	1.62 ± 0. 22	1.66 ± 0. 33
Significance	-	^{1}P =0.0001	^{2}P=0.0001	^{1}P=0.7301 ^{2}P=0.2749
GAG staining	2.86 ± 0. 38	2.74 ± 0. 43	2.60 ± 0. 36	2.56 ± 0. 52
Significance	-	^{1}P =0.4765	^{2}P=0.0994	^{1}P=0.8286 ^{2}P=0.3655

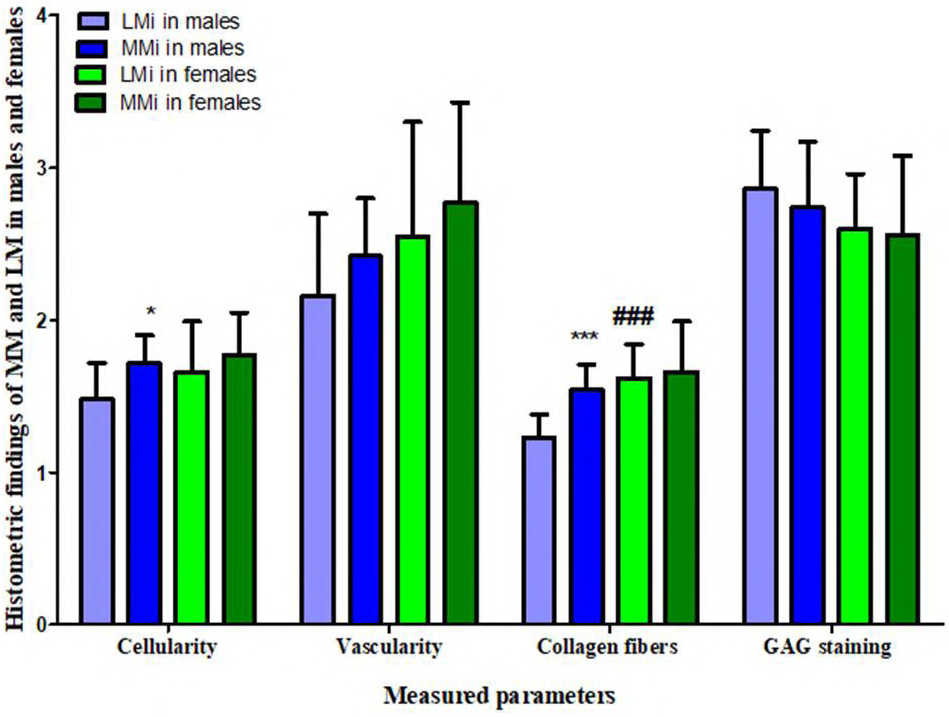

Fig. 8.- Histometric findings of MM versus LM in male and female cadavers: Data were expressed as mean ± SD, (*) represents the significance in LMi versus MMi while (#) is the significance male versus female.

lular modification manifested when comparing MMi between male and female groups.

In addition, MMi demonstrated an elevated degree of vascularity relative to LMi in both male and female groups, although without reaching statistical significance (*P*= 0.19 and 0.45 for male and female menisci, respectively). Likewise, a marginal yet statistically insignificant augmentation in vasculatrity was discerned in both female menisci when compared to their male counterparts. interestingly, and in line with the histological observations, a significant disruption in collagen fiber

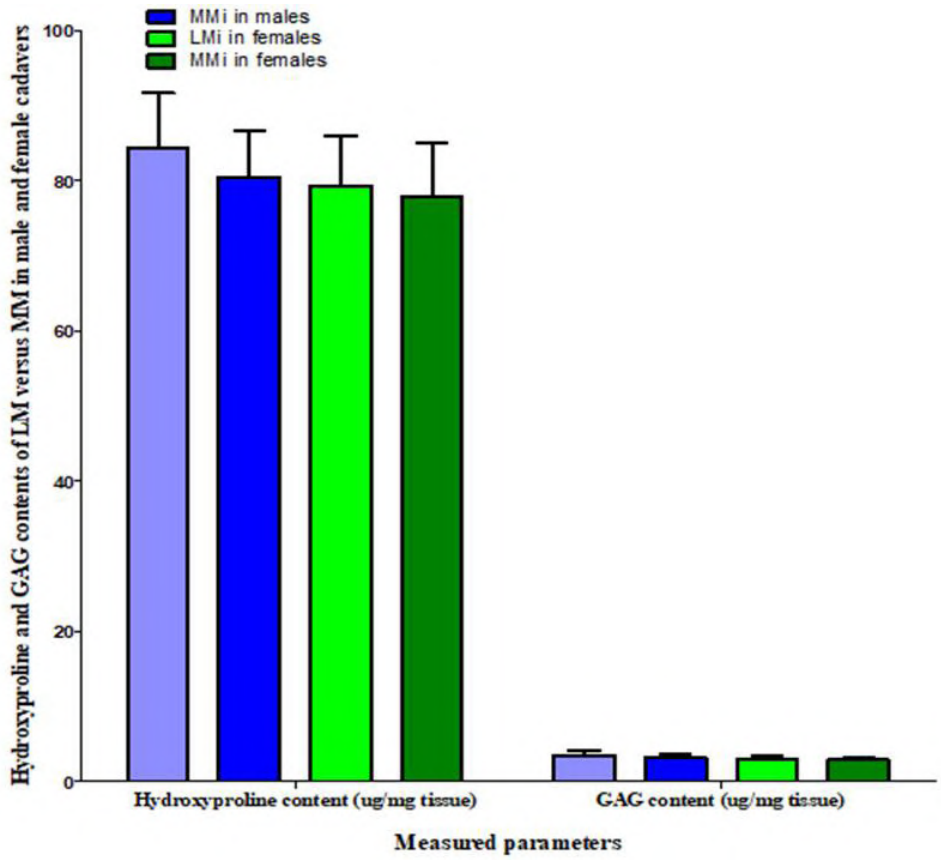

Fig. 9.- Hydroxyproline and GAG contents of LM versus MM in male and female cadavers: Data were expressed as mean ± SD. *: significance LMi versus MMi; #: significance male versus female.

Table 5. Hydroxyproline and GAG contents of LM versus MM in male and female cadavers.

Parameters	LMi (males)	MMi (males)	LMi (females)	MMi (females)
Hydroxyproline content (ug/mg tissue)	84.42 ± 7.24	80.55 ± 6.15	79.34 ± 6.68	77.82 ± 7.33
Significance	-	^{1}P =0.0783	^{2}P=0.0878	^{1}P=0.6008 ^{2}P=0.3337
GAG content(ug/mg tissue)	3.5 ± 0.66	3.2 ± 0.48	3.1 ± 0.34	2.9 ± 0.23
Significance	-	^{1}P =0.2162	^{2}P=0.0754	^{1}P=0.1056 ^{2}P=0.0637

arrangement was evident in MMi relative to LMi within male group (*P*= 0.001). This disarrangement was significantly more noticeable in the LMI of female participants when juxtaposed with the LMi of the male group (*P*= 0.001). A comparable albeit statistically insignificant pattern of disorganization was also observable in the MMI of the female cohort in comparison to their male counterparts.

Biochemical results

As shown in Table 5 and Fig. 9, the average hydroxyproline content was observed to be greater in LMi compared to MMi in both male and female menisci. Additionally, a higher hydroxyproline content was noted in the lateral and medial menisci of males when contrasted with their respective female counterparts. Although these observations

did not attain statistical significance, they aligned with the aforementioned microstructure examinations and histometric analyses of the meniscal tissues. The decreased hydroxyproline content in the female medial and lateral menisci suggested a lower collagen content within these tissues. Regarding GAG content, the mean values were also found to be higher in LMi compared to MMi in both male and female groups. The increased GAG content was also reflected on both male menisci when compared to those of the female group.

DISCUSSION

The microstructure of the extracellular matrix (ECM) components as well as cellularity and vasculatrity of the meniscus were specifically investigated in order to find and compare the structural disparities between the lateral and medial menisci within both male and female subjects. The motivation for conducting this study stemmed from the fact that the basic knowledge of meniscal cartilage architecture and gender-specific differences would be of great value, as it provides a deep understanding and a customized interpretation for the indications and outcome of regenerative, tissue repair and engineering strategies for the meniscal injuries (Cengiz et al., 2017; Pereira et al., 2019).

In the current study, the findings revealed distinctions in the organization and distribution of collagen fibers, cells, and blood vessels between male and female menisci. In both genders, the orientation of collagen fibers differed between the surface and deeper layers in all meniscal regions, with subtle variations observed in both lateral (LMi) and medial (MMi) menisci. This observation aligns with the studies by Makris et al. (2011) and Baker et al. (2011), which similarly highlighted the site- and depth-specific arrangement of collagen fibers that enhance the meniscus's ability to withstand tensile forces.

Despite the already established variability in collagen fiber orientation in MMi and LMi, we found that some female MMi displayed loose and unorganized collagen fibers distribution near the surfaces and in the inner region of the meniscus. This was further associated with irregular cellular arrangement and the presence of cell clusters. The above findings were supported by those of MT sections, which showed weaker reaction, particularly that of MMi in female group suggesting less collagen content. The density of the collagen fibers of the femoral side was even lower than the opposing tibial side in female menisci. Interestingly and consistent with the microstructural findings, quantitative image analysis of these sections showed significant disturbance in collagen fiber arrangement in female menisci when compared to those of male, which exhibited more dominance in the LMi among both groups.

We further extended our analysis to measure the amount of collagen in each meniscus by the assessment of hydroxyproline content. Collagen was found higher in male menisci when compared to female, and was once again higher in LMi than MMi in both male and female groups. Based on the above observations, we proposed that the menisci of female knees possess lower collagen content and less organization of ECM than those of male joints, and hence they are more prone to degeneration. It was proposed that knee degeneration is linked to the disruptions in particular structural elements rather than being a generalized effect across all histological components (Våben et al., 2020; Warnecke et al., 2020). Further investigation is therefore necessary to determine the precise relationship between these deficits or disarrangements and the overall degenerative process.

In addition, the previous work by Katsuragawa et al. (2010) showed that, in spite of the modest histological changes in the degenerating menisci, there was an enhanced mRNA expression of procollagen gene and ECM components. The increased expression of procollagen was not, however, proportional to protein content in the tissues, as the [^{3}H] proline incorporation was only modest in the degenerating menisci, suggesting an impaired collagen synthesis. We also assumed in this work that the overt matrix gene expression could be the result of a reparative response that occurred within OA menisci. The above findings and this speculation in fact explain why we noticed more collagen fiber content in LMi than MMi, irrespective of being more disarranged in female menisci. This difference between LMi and MMi is also reflected in knee biomechanics. It was

reported that MM covers 64% of the medial tibial plateau, while LM covers 84% of the lateral tibial plateau (Park et al., 2017). Thus, LM is responsible for most of the load transmission within the lateral compartment (up to 70%) while MM is responsible for 50% within its respective compartment. LM is more mobile, while the more static MM is known to play an additional secondary role as a joint stabilizer to resist the anterior tibial displacement (Masouros et al., 2008; Bloecker et al., 2012). Due to this spatial structural and conformation variability, the LM shows greater degree of activity and more stability compared with MMi (Swamy et al., 2018).

We found an alteration in the GAGs staining pattern of Safranin-O sections in which the high intensity areas of GAGs were more pronounced in the inner region of menisci in both genders. We also observed a regional and zonal variation of GAGs in the menisci. In accordance, it was documented that the inner two-thirds contained a relatively higher proportion of proteoglycans than the outer one-third (Melrose et al., 2005; Sanchez-Adams and Athanasiou, 2009).

The increased GAGs staining in the LMi vs MMi in female group was confirmed by the biochemical evaluation of GAGs content in the corresponding menisci. In contrast, GAGs histological findings seemed contradictory to those of biochemical results in the male group. Despite these discrepancies, the total amount of GAGs measured in female menisci was less than that of male samples. In fact, any changes in the GAGs synthesis and organization can lead to extracellular framework perturbation and, consequently, render the female menisci more vulnerable to degeneration. In line with our observation, López-Franco et al. (2016) reported a tendency towards proteoglycan reduction in human OA menisci. Also, it was stated that GAGs play an important role in the hydration of meniscal tissue and its viscoelastic ability to resist compression and loading. Accordingly, regional variations can be observed in terms of viscoelastic properties (Mahmood et al., 2019; Murphy et al., 2019).

In the current study, the examination of the meniscal cellularity revealed the presence of spindle-shaped fibroblasts-like cells without lacunae at peripheral two-thirds, which decreased as we extend toward the inner concave margin of the menisci. We also noticed round or oval cells lying in the lacunae resembling cartilage cells, which were interspersed toward the inner margin. Our findings were in accordance with previous studies done by Niu et al. (2016) and Cengiz et al. (2017). They reported the distribution of the meniscal cells as three different morphologies; the superficial zone contains fusiform cells directly located beneath the tissue surface; the outer one-third contains fibroblast-like cells with elongated morphology; and the inner two-thirds contain fibrochondrocytes with polygonal or oval morphology.

Our histometric analysis showed alteration in term of cellular disorganization and increased cell populations in male MMi versus LMi. It is also worth noting the increase in cellular disarray in female MMi and LMi, as opposed to their male counterparts, although this increase was not statistically significant. Also, a progressive decline in cell density was observed from the outer vascular zones to the inner avascular zones in both LMi and MMi.

In accordance with our findings, it was reported that different types of cells are distributed in a regional-specific manner and account for the double nature of the meniscus. In the outer zone, fibrocyte-like cells reflect the fibrous pattern of the meniscus and contribute to the healing capability, while in the inner zone chondrocyte-like cells are responsible for the cartilaginous-like behavior of the tissue (Sanchez-Adams and Athanasiou, 2009; Makris et al., 2011; Osawa et al., 2013; Pereira et al., 2014; Chahla et al., 2021).

In this study, we noticed that both LMi and MMi displayed some differences with respect to vascularity, in which the periphery of MM is slightly more vascularized (10–30%) than LM (10-25%). Correspondingly, Gupta et al. (2018) noted that the highest vasculature rates were recorded in the anterior horn of the MMi, ranging from 22% to 83% (with an average of 40%), while the meniscal body exhibited vascular indices spanning from 20% to 62% (averaging at 30%). In the case of the LMi, heightened vasculature was particularly evident in two key areas: the anterior horn, displaying rates of 18% to 95% (with an average of 48%),

and the posterior horn, registering rates ranging from 22% to 52% (averaging at 31%).

Also, previous studies have mentioned that each meniscus is classified into three zones: vascularized, intermediate, and avascular. Therefore, tears within the vascular zone have a higher healing rate comparing with that in avascular zone whereas the white–white zone is more susceptible to degenerative and posttraumatic lesions and the damaged menisci have limited repair capacity in the avascular region of meniscus (Majeed et al., 2015; Cinque et al., 2019).

At the end, this study provides valuable information about the microstructure of medial and lateral menisci, including collagen fiber orientation, cell arrangement, and blood vessel distribution, shedding light on their histological and biochemical differences in males and females. However, our study utilizes cadaveric samples, which might not fully represent the dynamic nature of living tissue. Also, while comparing between males and females is valuable, the study does not explicitly discuss why gender differences might exist or how hormonal or physiological factors could contribute to the observed variations. Moreover, although this study offers valuable insights into the histological and biochemical characteristics of the menisci, it does not directly correlate these findings with clinical conditions or pathological lesions, potentially limiting the practical implications. Nevertheless, this study could be further followed by conducting additional research that discuss the potential functional implications and integrates the clinical correlations of its findings.

CONCLUSION

Our work provides the first line of entry into the comparative analysis of knee menisci. The histological and histometric data gained here are crucial to give additional information on microstructure of MMi and LMi in males and females. This is necessary for the correlation with different meniscal injuries and degeneration that can help in reconstructive and surgeries of knee. Taken together, we hypothesised that the main structural features cellularity, collagen structure and vascularity exhibited differences in MMi and LMi in males and females, which are expected to influence the repair processes.

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