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Microscopic Assessment of Meniscal Cellularity in Knee Osteoarthritis patients undergoing total knee replacement surgery.

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Abstract

Background: The human knee menisci play crucial roles in joint function, including load distribution and shock absorption. Degenerative changes in these structures, often associated with osteoarthritis (OA), can significantly impact joint health and mobility. Understanding the histological alterations in knee menisci during OA progression is essential for elucidating disease mechanisms and guiding therapeutic interventions.

Material and Methods: This cross-sectional study investigated histological changes in both medial and lateral menisci from 30 knee joints from patients undergoing total knee replacement (TKR) due to knee OA. Meniscal tissues were obtained intraoperatively, fixed in formalin, processed, and stained with hematoxylin and eosin for microscopic evaluation. Menisci were graded on a scale from Grade 0 to Grade 3 based on a specific OA meniscus cellularity grading system. Statistical analyses, including independent samples t-tests and paired samples t-tests, were employed to assess differences in meniscal cellularity between sexes and between medial and lateral compartments.

Results: The medial meniscus showed comparable mean cellularity scores between males (1.27 ± 0.80) and females (1.53 ± 1.20) ($p = 0.339$), indicating similar histological changes regardless of sex. In contrast, significant sex-based differences were observed in the lateral meniscus, with females (1.27 ± 1.03) exhibiting higher cellularity scores compared to males (0.53 ± 0.52) ($p = 0.023$). Within-individual analysis revealed significantly higher cellularity in the medial meniscus (1.40 ± 1.00) compared to the lateral meniscus (0.90 ± 0.88) ($p = 0.007$).

Conclusion: This study underscores distinct histological patterns of meniscal cellularity in knee OA, emphasizing sex-specific variations and compartmental differences. The findings highlight the potential utility of histopathological assessments in refining clinical management strategies and developing targeted interventions for knee OA based on sex and meniscus type. Further research with larger cohorts and longitudinal designs is warranted to validate these findings and explore additional histological parameters influencing meniscal integrity in knee OA progression.

Keywords: Knee menisci, Osteoarthritis, Histopathology, Meniscal cellularity, Total knee replacement, Sex differences.

Introduction

The human knee menisci, located between the femur and tibia, serve several vital functions such as load distribution, shock absorption, joint stability, and lubrication.[1] Each joint has two menisci – the medial and lateral menisci located on the tibial plateau. Damage to the menisci, either through trauma or degeneration, can disrupt its functions, often leading to the development of osteoarthritis (OA). OA is a degenerative joint disease characterized by the breakdown of cartilage, subchondral bone changes, synovial inflammation, and osteophyte formation.[2] OA of knee joint is most often reported in old age which could be due to degeneration or ageing.[3] Knee OA causes chronic pain, stiffness, and swelling, leading to reduced mobility and difficulty in performing daily activities. It often results in joint deformity, decreased quality of life, and may require surgical interventions like total knee replacement. Additionally, it can increase the risk of obesity and cardiovascular issues.

Meniscal changes due to aging often involve abnormal matrix organization and cellularity. Studies have reported degenerative findings such as fibrocartilaginous separation of the matrix, extensive fraying, tears, and calcification.[4-6] However, most grading systems for meniscus pathology are MRI-based, focusing on tears and grades of mucoid degeneration.[7-10] The molecular mechanisms driving the onset and progression of meniscal degeneration are not fully understood, and there is limited knowledge about meniscal aging at the microscopic level. Most of the current understanding of microstructural changes in the osteoarthritic meniscus comes from animal studies.[11-14]

The histopathological study of the meniscus holds significant potential for understanding disease mechanisms, predicting clinical outcomes, and guiding management strategies. Microscopic evaluation is thus an important tool for assessing meniscal pathology. This study aims to evaluate the histological changes in the knee menisci (both medial and lateral) in patients clinically diagnosed with osteoarthritis (OA) who are planned for total knee replacement (TKR). By examining human meniscus changes caused by knee OA, the study seeks to identify the histological alterations in the meniscus cellularity which can provide structural evidence for the cause of knee OA.

Materials and methods

Study Design:

This hospital based cross-sectional study was undertaken to identify the histological changes in meniscal cellularity that can provide structural evidence for the development of knee osteoarthritis. Before the commencement of the study, ethical clearance was obtained from the Institutional Ethical Committee (AIIMS/MG/IEC/2023-24/46). Demographic data including age, sex, height, weight, and BMI was be obtained through the review of medical records of each participant.

Study Setting:

The study was conducted in a tertiary care public hospital over a period of six months in 2023-2024 by the Department of Orthopedics and Department of Anatomy, and, All India Institute of Medical Sciences, Mangalagiri, India.

Sample Size:

A total of 30 knee specimen involving both medical and lateral menisci were included in the study. The sample size was calculated considering power of the study as 90% power with 95% confidence interval. Written informed consent was obtained from all TRK patients before including them in this study.

Inclusion Criteria:

1. Patients undergoing total knee replacement surgery due to osteoarthritis.

Exclusion Criteria:

1. Patients who have undergone partial of total meniscectomy.
2. Patients with secondary knee arthritis.
3. Patients who had undergone any surgical interventions or procedures to the knee.

Sample Collection and Preparation

Both the medial and lateral menisci were carefully excised during the surgical procedure. The excised meniscal tissues were immediately placed in 10% formalin solution to fix the tissues and preserve their structural integrity. Each meniscus was sectioned vertically in the middle to include both peripheral rim to center and were subjected to routine tissue processing and staining.[15]

Dehydration, Clearing and Embedding

Following fixation, the meniscal samples were subjected to a series of dehydration steps using ascending grades of alcohols. Each step in the series ensured the gradual removal of water from the tissue, which is necessary to facilitate the infiltration of paraffin wax. Dehydration was carefully monitored to avoid tissue shrinkage or hardening. Once dehydration was complete, the samples were placed in xylene which serves as a clearing agent, replacing the alcohol within the tissue and making it transparent. After clearing, the meniscal tissues were impregnated with molten paraffin wax at a temperature of approximately 58-60°C. The paraffin-embedded tissues were then allowed to cool and solidify, forming a paraffin block.

Sectioning and Staining:

The paraffin-embedded meniscal tissues were sectioned using a microtome to get thin sections of tissue of 4-5 micrometers in thickness. These thin sections are essential for detailed histological analysis as they allow light to pass through the tissue when viewed under a microscope. Each section was carefully collected on glass slides, ensuring that they were free from folds or tears. The tissue sections were stained using the conventional hematoxylin and eosin (H&E) staining method. Hematoxylin stains the cell nuclei blue, while eosin stains the cytoplasm and extracellular matrix pink.

Microscopic Examination:

The stained slides were observed under a light microscope. Histological parameters were studied and each menisci was graded from Grade 0 to Grade 3 based on the meniscus cellularity based to OA menisci grading score.[16] The findings were tabulated and coded using a master sheet in Microsoft Excel.

Statistical analysis:

An independent samples t-test was used to determine if there was a significant difference in the mean cellularity scores of the medial meniscus between males and females and the mean cellularity scores of lateral meniscus between males and females. A paired samples t-test was used to determine if there was a significant difference in the mean cellularity scores between the medial and lateral menisci within the same subjects. Statistical analysis was preformed using GraphPad Prism software. A p-value ≤0.05 was considered statistically significant.

Results

A total of 30 individuals scheduled for total knee replacement (TKR) surgery were included in this study, comprising an equal distribution of 15 males and 15 females. The average age of the participants was 59.7 ± 4.1 years. The mean height of male participants was 168.2 ± 7.3 cm, while for females, it was 155.6 ± 5.2 cm. The mean BMI for males was 26.6 ± 3.3, and for females, it was 29.6 ± 3.3. Histological parameters were assessed, and each meniscus was categorized on a scale from Grade 0 to Grade 3 according to a grading system specific to osteoarthritis-related changes in meniscal cellularity (Figure-1).

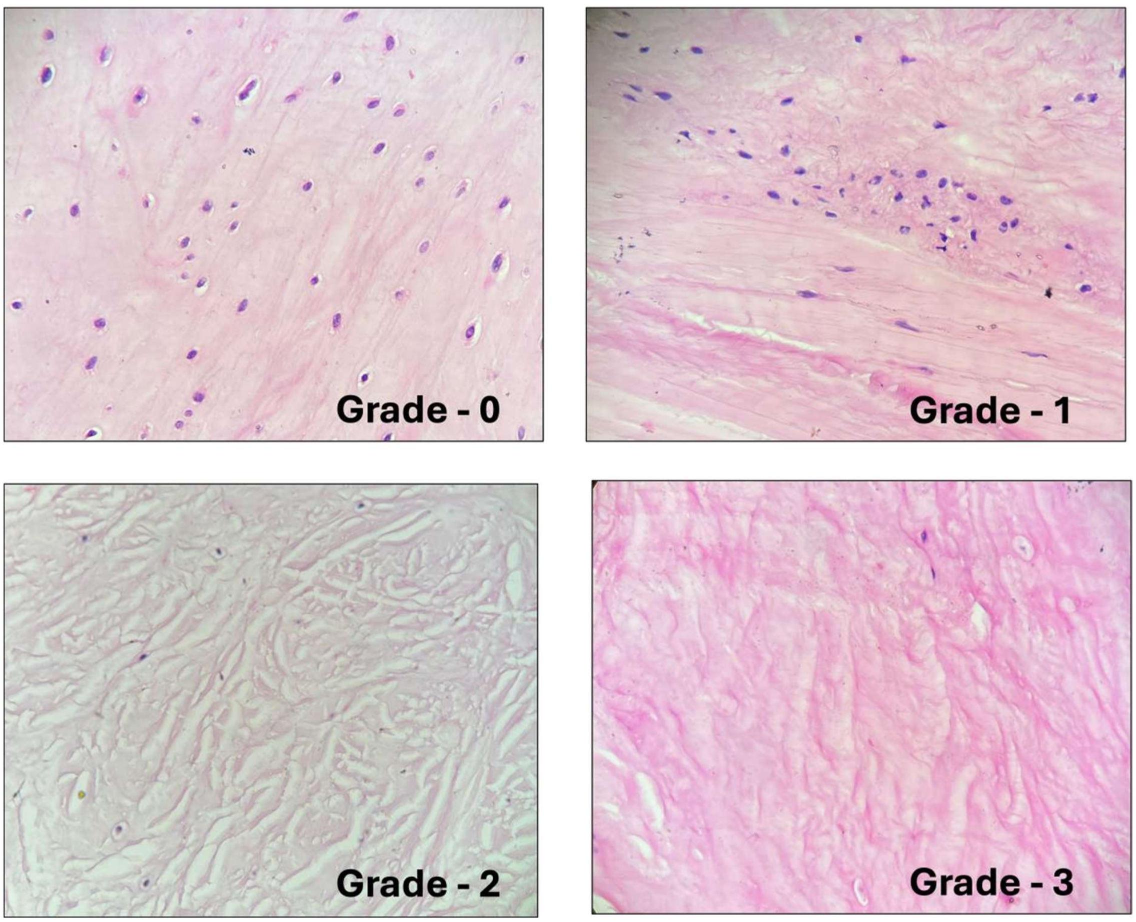

Figure-1: Histological assessment of meniscus cellularity: (A) Normal cell distribution, Grade 0. (B) Diffuse hypercellularity, Grade1. (C) Meniscus tissue shows hypo- to acellular regions, Grade 3 (D) Hypocellular meniscus tissue, Grade 3. Hematoxylin & Eosin, 40x.

Meniscal Cellularity - Medial Meniscus

The analysis of the medial meniscus cellularity revealed a mean cellularity score of 1.27 ± 0.80 in males and 1.53 ± 1.20 in females. An independent samples t-test showed no statistically significant difference between the mean cellularity scores of males and females (t = -0.973, p = 0.339), indicating similar cellularity alterations in the medial menisci of both sexes. (Table-1)

Group	N	Mean meniscus cellularity	Std. Deviation	P value
Medial meniscus (Male)	15	1.27	0.80	0.339
Medial meniscus (Female)	15	1.53	1.20	

Table 1: Comparison of Meniscus Cellularity Between Male and Female Medial Meniscus

Meniscal Cellularity - Lateral Meniscus

In contrast, the lateral meniscus cellularity showed a significant difference between sexes. The mean cellularity score for males was 0.53 ± 0.52, whereas for females, it was 1.27 ± 1.03. The independent samples t-test revealed a statistically significant difference ($t = -2.405$, $p = 0.023$), suggesting that female participants exhibited higher cellularity changes in the lateral meniscus compared to males. (Table-1)

Group	N	Mean meniscus cellularity	Std. Deviation	P value
Lateral meniscus (Male)	15	0.53	0.52	**0.023**
Lateral meniscus (Female)	15	1.27	1.03	

Table 2: Comparison of Meniscus Cellularity Between Male and Female Lateral Meniscus

Medial vs. Lateral Meniscus

A paired samples t-test was conducted to compare the cellularity scores of the medial and lateral menisci within the same individuals. The medial meniscus had a mean cellularity score of 1.40 ± 1.00, while the lateral meniscus had a mean score of 0.90 ± 0.88. The test indicated a statistically significant difference ($t = 2.786$, $p = 0.007$), suggesting that cellularity changes were more pronounced in the medial meniscus compared to the lateral meniscus in the context of knee osteoarthritis. (Table-3)

Group	N	Mean meniscus cellularity	Std. Deviation	P value
Medial meniscus (Overall)	30	1.40	1.00	**0.007**
Lateral meniscus (Overall)	30	0.90	0.88	

Table 3: Comparison of Meniscus Cellularity Between Medial and Lateral Meniscus

The findings from this study highlight significant differences in meniscal cellularity based on sex and meniscus type. While the medial meniscus showed no significant sex-based differences in cellularity, the lateral meniscus cellularity was significantly higher in females. Additionally, within individuals, the medial meniscus exhibited greater cellularity changes compared to the lateral meniscus. These results underscore the importance of considering both sex and meniscus type in the histological evaluation of knee osteoarthritis, as they may influence the structural evidence and progression of the disease.

Discussion

The histopathological examination of knee menisci in patients undergoing total knee replacement (TKR) surgery due to osteoarthritis (OA) provides valuable insights into the structural changes associated with this degenerative joint disease. In this study, we investigated the histological alterations in both medial and lateral menisci, focusing on differences in cellularity between sexes and meniscus types.

Our findings revealed distinct patterns of meniscal cellularity alterations in relation to sex and meniscus location. Specifically, we observed that while the medial meniscus exhibited similar mean cellularity scores between males (1.27 ± 0.80) and females (1.53 ± 1.20), no statistically significant difference was found ($p = 0.339$). This suggests that in the context of knee OA, cellularity changes in the medial meniscus are comparable between sexes. These results align with previous studies [17-19] indicating that certain degenerative changes in the meniscus may not show significant sex-related variations in histopathological assessments.

Conversely, our study identified significant differences in cellularity patterns within the lateral meniscus. Female participants demonstrated higher mean cellularity scores (1.27 ± 1.03) compared to males (0.53 ± 0.52), with a statistically significant difference observed ($p = 0.023$). This disparity highlights potential sex-specific variations in the pathological processes affecting the lateral meniscus during knee OA progression. Such findings underscore the importance of considering sex as a variable in histological studies of knee menisci, as it may influence disease mechanisms and outcomes.

Moreover, our study evaluated the comparative cellularity changes between the medial and lateral menisci within the same individuals. We found that the medial meniscus exhibited a significantly higher mean cellularity score (1.40 ± 1.00) compared to the lateral meniscus (0.90 ± 0.88), with a p-value of 0.007. This intra-individual difference suggests that in the context of knee OA, the medial meniscus may undergo more pronounced cellular alterations than the lateral meniscus. Such findings are consistent with clinical observations where medial compartment OA often presents with more severe symptoms and structural changes compared to the lateral compartment.

The implications of these histological findings extend to understanding the progression and management of knee OA. Meniscal cellularity, as assessed through histopathological analysis, provides critical structural evidence that complements clinical assessments and imaging modalities. The identification of sex-specific differences in lateral meniscal cellularity underscores the need for personalized approaches in OA management, potentially targeting specific pathological processes based on sex-related variations.

Limitations of our study include the relatively small sample size and the cross-sectional design, which restricts causal inference and longitudinal assessment of disease progression. Future research could benefit from larger cohorts and longitudinal studies to validate these findings and explore additional histopathological parameters influencing meniscal integrity and function in knee OA.

Conclusion

Our study contributes to the growing body of literature on the histological characterization of knee menisci in OA, emphasizing the importance of sex-specific considerations and differential analysis between medial and lateral compartments. By elucidating these histopathological nuances, our findings pave the way for enhanced diagnostic strategies and targeted therapeutic interventions aimed at preserving meniscal function and mitigating the progression of knee OA.

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