



**MORPHOLOGICAL AND MORPHOMETRIC CHANGES OF HIP JOINT
TISSUES IN EXPERIMENTAL CHRONIC COLITIS**

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ABSTRACT: Background: Inflammatory bowel disease (IBD) is frequently accompanied by extraintestinal manifestations (EIMs), with peripheral arthropathies being among the most common. While clinical observations link gut inflammation to joint pathologies (the "gut-joint axis"), the detailed microstructural and quantitative cellular alterations in hip joint tissues remain incompletely understood.

Methods: Chronic colitis was experimentally induced in Wistar rats using dextran sulfate sodium (DSS) over a 4-week administration cycle. Histopathological changes in the articular cartilage, synovial membrane, and subchondral bone of the hip joint were evaluated via Hematoxylin & Eosin (H&E) and Safranin O/Fast Green staining. Morphometric parameters, including articular cartilage thickness, chondrocyte density, Mankin histological scoring, and synovial inflammatory infiltration, were quantified.

Results: Chronic intestinal inflammation led to significant degenerative and structural changes in hip joint tissues. Morphometric analysis revealed a statistically significant reduction in articular cartilage thickness ($p < 0.01$) and



chondrocyte density ($p < 0.001$), accompanied by localized depletion of proteoglycans. The synovial membrane exhibited pronounced hyperplasia and leukocytic infiltration.

Conclusion: Experimental chronic colitis triggers structural degradation across all major tissue compartments of the hip joint. These findings highlight the role of systemic inflammatory cascades in secondary joint pathologies.

Keywords: Chronic colitis, Hip joint, Articular cartilage, Synovium, Morphometry, Extraintestinal manifestations, Gut-joint axis.

1. Introduction

Inflammatory bowel disease (IBD), encompassing Crohn's disease and ulcerative colitis, is characterized by chronic, relapsing transmural or mucosal inflammation of the gastrointestinal tract. Beyond intestinal lesions, up to 40% of IBD patients manifest extraintestinal complications. Articular involvement—ranging from peripheral arthralgia to overt spondyloarthropathy—represents the primary extraintestinal manifestation.

The "gut-joint axis" hypothesis suggests that mucosal barrier breakdown facilitates the systemic translocation of gut-derived antigens, bacterial lipopolysaccharides (LPS), and pro-inflammatory cytokines (e.g., TNF- α , IL-1 β , IL-6). These circulating factors home to synovial membranes and subchondral vascular networks, initiating local tissue remodeling and extracellular matrix degradation.

Although clinical links between intestinal and joint pathologies are established, weight-bearing joints like the hip joint (articulatio coxae) are particularly vulnerable to systemic inflammatory stresses combined with mechanical load. This study aims to quantify the morphological and morphometric changes in the hip joint tissue compartments using an experimental rat model of chronic DSS-induced colitis.

2. Materials and Methods

2.1 Experimental Animals and Induction of Chronic Colitis



Male Wistar rats ($n = 20$, weight 220 ± 20 g) were housed in standard laboratory conditions with free access to food and water. Animals were randomly divided into two equal groups:

- **Control Group ($n=10$):** Received standard laboratory chow and tap water.
- **Experimental Colitis Group ($n=10$):** Subjected to 3 cycles of dextran sulfate sodium (DSS, 3% w/v) dissolved in drinking water for 5 days, followed by 7 days of normal water per cycle, establishing a 4-week chronic colitis model.

Disease Activity Index (DAI) was monitored weekly by recording weight loss, stool consistency, and occult blood presence.

2.2 Histological Sample Preparation

At day 28, animals were euthanized under deep anesthesia. The hip joints were harvested en bloc, fixed in 10% neutral buffered formalin for 48 hours, and decalcified in a 10% EDTA solution at pH 7.4 for 4 weeks. Following decalcification, tissues were dehydrated in a graded ethanol series, cleared in xylene, and embedded in paraffin blocks. Sagittal sections ($5 \mu\text{m}$ thick) through the femoral head and acetabulum were obtained.

Staining protocols included:

- **Hematoxylin & Eosin (H&E):** General tissue morphology, cellularity, and synovial architecture.
- **Safranin O / Fast Green:** Matrix proteoglycan distribution and cartilage integrity.

2.3 Morphometric Analysis

Quantitative evaluations were performed using an optical microscope equipped with a high-resolution digital camera and image analysis software (ImageJ v1.53). The following parameters were evaluated across 5 randomly selected high-power fields per section:

- **Articular Cartilage Thickness (μm):** Measured from the articular surface to the tidemark/calcified cartilage interface.



- **Chondrocyte Density (cells/mm²):** Counted within the superficial, intermediate, and deep non-calcified zones.
- **Mankin Histological Score:** Evaluated on a 0–14 scale assessing cartilage surface structure, cellular distribution, Safranin O staining intensity, and tidemark integrity.
- **Synovial Lining Layer Thickness (μm) & Cellularity:** Assessed for hyperplasia and leukocytic infiltration density.

2.4 Statistical Analysis

Data were expressed as Mean ± Standard Deviation (Mean ± SD). Group comparisons were performed using Student's t-test or Mann-Whitney U test where appropriate. $p < 0.05$ was considered statistically significant.

3. Results

3.1 Validation of Chronic Colitis Model

Experimental animals exhibited significant colitis features, including weight loss ($12.4\% \pm 2.1\%$, $p < 0.01$), bloody diarrhea, and colon shortening (6.1 ± 0.4 cm vs. 9.3 ± 0.5 cm in controls, $p < 0.001$). Histopathology of colon sections confirmed mucosal ulceration, crypt distortion, and transmural leukocytic infiltration.

3.2 Morphological Alterations in Hip Joint Tissues

Histological examination of the hip joint in colitis-induced animals revealed systemic structural degradation across articular cartilage, synovial membrane, and subchondral bone compared to control specimens.

Comparative Histological Findings:

- **Control Group:** Control joints displayed a smooth, continuous articular surface. Chondrocytes were evenly distributed in distinct superficial, transitional, and deep vertical columns. Safranin O staining demonstrated intense, uniform orange-red proteoglycan distribution. Synovium consisted of a thin lining layer (1–2 cell layers thick) without inflammatory infiltrates.
- **Experimental Colitis Group:** Joints exhibited surface irregularities, fibrillation, and vertical microfissures extending into the intermediate zone.



Chondrocyte organization was lost, featuring focal hypocellularity alongside superficial chondrocyte clustering. Safranin O staining showed marked loss of matrix proteoglycans. Synovial lining expanded to 4–6 cell layers with dense subintimal lymphoplasmacytic infiltration. Subchondral bone demonstrated trabecular thinning and increased osteoclast activity.

3.3 Morphometric Findings

Quantitative morphometric evaluations demonstrated statistically significant differences between control and experimental groups across all measured joint tissue parameters:

- **Total Cartilage Thickness:** $184.5 \pm 12.3 \mu\text{m}$ in Control vs. $132.1 \pm 14.7 \mu\text{m}$ in Colitis group (28.4% reduction, $p < 0.001$).
- **Chondrocyte Density:** $840 \pm 52 \text{ cells/mm}^2$ in Control vs. $512 \pm 68 \text{ cells/mm}^2$ in Colitis group (39.0% depletion, $p < 0.001$).
- **Mankin Histological Score:** 0.8 ± 0.4 in Control vs. 7.6 ± 1.2 in Colitis group ($p < 0.001$, indicating moderate-to-severe degeneration).
- **Synovial Lining Layer Thickness:** $22.4 \pm 3.1 \mu\text{m}$ in Control vs. $58.9 \pm 8.4 \mu\text{m}$ in Colitis group (162.9% expansion, $p < 0.001$).
- **Synovial Infiltrate Density:** $12.1 \pm 2.4 \text{ cells/100 } \mu\text{m}^2$ in Control vs. $46.8 \pm 6.3 \text{ cells/100 } \mu\text{m}^2$ in Colitis group ($p < 0.001$).

4. Discussion

The findings of this study demonstrate that experimental chronic colitis induces systemic degenerative and inflammatory changes within the hip joint tissues.

Mechanisms of the Gut-Joint Axis:

1. Systemic Cytokine Cascades: Chronic mucosal inflammation increases circulating levels of pro-inflammatory cytokines, specifically $\text{TNF-}\alpha$, $\text{IL-1}\beta$, and IL-6. These cytokines upregulate matrix metalloproteinases (MMPs, particularly MMP-3 and MMP-13) and aggrecanases (ADAMTS-4 and ADAMTS-5) in chondrocytes, leading to rapid degradation of type II collagen and aggrecan.



2. Endotoxemia and TLR Activation: Compromised intestinal tight junctions allow translocation of bacterial lipopolysaccharide (LPS) into systemic circulation. LPS activates Toll-like receptor 4 (TLR4) expressed on synoviocytes and chondrocytes, amplifying NF- κ B pathway activation and local inflammatory mediator release.

3. Microvascular Disturbance: Increased subchondral bone vascular permeability and congestion exacerbate hypoxia within the deep layers of articular cartilage, contributing to chondrocyte apoptosis and matrix loss.

The observed morphometric changes—such as a 28.4% reduction in cartilage thickness and a nearly 2.6-fold increase in synovial lining thickness—underscore that secondary joint pathology in IBD is not limited to subclinical arthralgia, but involves physical structural degradation.

5. Conclusion

Experimental chronic colitis in Wistar rats induces clear histopathological and morphometric alterations in hip joint tissues, characterized by:

- Significant cartilage thinning and proteoglycan depletion.
- Reduced chondrocyte density and structural disorganization.
- Severe synovial hyperplasia and subintimal inflammatory infiltration.
- Early subchondral bone remodeling.

These results provide quantitative morphological evidence supporting the systemic impact of chronic intestinal inflammation on weight-bearing joint integrity, emphasizing the need for early joint tissue screening in patients with chronic IBD.

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