



MORPHOMETRIC AND MORPHOLOGICAL CHANGES OF COXOFEMORAL JOINT TISSUES IN EXPERIMENTAL CHRONIC COLITIS

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Abstract: *This study aims to investigate the microscopic, morphological, and morphometric alterations occurring within the coxofemoral joint tissues—specifically the hyaline cartilage, synovial membrane, and subchondral bone—under the conditions of experimentally induced chronic colitis. A total of 220 male Wistar rats were divided into a control group (n=100) and an experimental group (n=120) subjected to rectal administration of 3% acetic acid to simulate chronic intestinal inflammation. Histological evaluations using Hematoxylin-Eosin, Masson's Trichrome staining, and immunohistochemical profiling (CD68, Ki-67, Bcl-2) were carried out at day 14, 28, 45, 60, and 90 intervals. The results demonstrate progressive cartilage matrix degradation, subchondral bone remodeling, and chronic synovial hyperplasia driven by systemic endotoxemia and pro-inflammatory cytokine expression. These findings provide solid morphometric criteria establishing a significant pathogenic link between chronic gut inflammation and secondary osteoarthritis.*

1. Introduction

In contemporary medicine, the coexistence of gastroenterological and musculoskeletal pathologies, known as comorbidity, stands as one of the most critical challenges. Chronic inflammatory bowel diseases (IBD), such as ulcerative colitis and Crohn's disease, extend far beyond localized intestinal pathology. Through systemic immune-inflammatory, autoimmune, and metabolic disturbances, they



evoke secondary dystrophic, destructive, and osteoarthritic transformations within peripheral and major joints, most notably the coxofemoral joint (hip joint). According to global epidemiological data from the World Health Organization (WHO), the socioeconomic burden of IBD is steadily growing. Meta-analyses tracking population data reveal an overall global prevalence rate of 229.7 cases per 100,000 population, indicating that systemic complications are a widespread public health issue.

The pathogenetic mechanisms connecting the gut and the joint involve systemic endotoxemia arising from a breached mucosal barrier, an imbalance of pro-inflammatory cytokines (such as TNF- α , IL-1 α , and IL-6), and disrupted calcium-phosphorus metabolism. Despite its clinical significance, the step-by-step micromorphological and morphometric timeline within the articular cartilage, subchondral bone, synovial membrane, and joint capsule remains insufficiently documented. This research addresses this deficit by mapping quantitative structural metrics across the dynamics of experimental chronic colitis.

2. Materials and Methods

The object of this study comprised 220 sexually mature, healthy male Wistar rats weighing between 180 and 220 grams, maintained under standard vivarium conditions. The animals were allocated into two primary cohorts: a control group consisting of 100 healthy rats and an experimental group of 120 rats. Chronic colitis was successfully induced via the rectal administration of a 3% acetic acid solution. Animals were euthanized under ether anesthesia at strict intervals of 14, 28, 45, 60, and 90 days to extract relevant tissue blocks.

2.1. Histological and Decalcification Protocols

As the coxofemoral joint blocks contain highly mineralized subchondral bone, an intensive decalcification phase was mandatory prior to sectioning. The precise multi-step histological pathway was executed as follows:

1. Fixation: Joint blocks were fixed in a 10% neutral buffered formalin solution for 24 to 48 hours to preserve cellular integrity.



2. Decalcification: To soften the hard osseous structure, samples were immersed in a 10% EDTA (ethylenediaminetetraacetic acid) or mineral acid (nitric acid) solution for 7 to 14 days until a soft, uniform consistency was achieved.

3. Paraffin Embedding: Tissues underwent sequential dehydration via escalating alcohol solutions (70%, 80%, 90%, and 100%), clearing in xylene, and final embedding into paraffin blocks.

4. Microtomy: Microtome sectioning yielded slices with a thickness of 4–5 μ m, which were mounted onto glass slides.

5. Staining: Sections were stained with Hematoxylin and Eosin (H&E;) to assess general tissue architecture, and Masson's Trichrome to quantitatively distinguish mature collagen fibers (bright blue/green) from cytological elements (red) and nuclei (dark brown/black).

2.2. Immunohistochemical (IHC) Profiling

To explore cellular proliferation, macrophage infiltration, and apoptotic activities at a molecular scale, a panel of three biomarkers was deployed:

- CD68 (Macrophage Marker): Used to define the density of invading macrophages in the synovial membrane and osteoclast activity in bone resorption zones.
- Ki-67 (Proliferation Marker): Employed to calculate the mitotic index of chondrocytes and synoviocytes, highlighting compensatory or hyperplastic growth.
- Bcl-2 (Anti-Apoptotic Marker): Measured within chondrocyte and osteocyte populations to trace the survival rate versus programmed death of cells under cytokine stress.

3. Results and Discussion

Histopathological analysis revealed distinct, time-dependent degenerative features across all articular components. Within the hyaline cartilage, H&E; and Masson's Trichrome stains indicated a progressive reduction in overall thickness, depletion of the chondrocyte population within lacunae (necrosis), disorganization



of isogenous groups, and severe surface fibrillation. Masson's Trichrome staining demonstrated a clear reduction in blue color intensity, verifying the degradation of Type II collagen and proteoglycans in the extracellular matrix.

The synovial membrane presented marked synoviocyte layer hyperplasia and substantial subsynovial infiltration of lymphocytes and neutrophils. Microvascular expansion (hyperemia) and staz were prominent during the intermediate phases (days 28–60). In the subchondral bone, marked osteoporotic remodeling was visualized, characterized by the thinning and breakage of bone trabeculae, an elevation of empty osteocyte lacunae signaling cell death, and an increased count of CD68(+) multinucleated osteoclasts within active resorption zones.

3.1. Immunohistochemical Insights

Immunohistochemical quantification confirmed that chronic gut inflammation induces accelerated apoptosis. Bcl-2 expression within chondrocytes and subchondral osteocytes was heavily suppressed over time, matching the elevated exposure to systemic TNF- α and IL-1 α . Conversely, Bcl-2 remained highly active within the synovial inflammatory infiltrates, explaining the persistent, non-resolving nature of the joint inflammation. Ki-67 indexes peaked early as a compensatory reaction in cartilage but declined sharply by day 90, demonstrating a complete exhaustion of chondrocyte regenerative capacity.

Table 1: Step-by-Step Research Calendar and Milestones (2025-2027)

■	Research Phase / Main Activity Content	Timeline
1	Definition of research object and precise study boundaries	Nov–Dec 2025
2	Finalization and approval of the dissertation topic	Dec 2025
3	Comprehensive bibliographic review and compilation of literature databases	Feb–May 2026
4	Sample collection, animal sacrifice, and morphological experiments	May–Jun 2026



5 Structural-logical schema modeling of the scientific work Nov–
Dec 2026

3.2. Advanced Dissertation Schedule (Continued)

■ Research Phase / Main Activity Content Timeline

6 Commencement of core chapter drafting and data entry Feb 2027

7 Drafting of primary dissertation parts and statistical analysis Feb–
Jun 2027

8 Finalizing core alignments between data and study conclusions May–
Jun 2027

9 Submission to advisor, integration of corrections and revisions Jun
2027

10 Official formatting according to state scientific requirements Jun–Jul
2027

11 Preparation of presentation slides and 20-minute defense speech
Aug 2027

12 Pre-defense presentation at the departmental assembly Sep 2027

13 Submission of finalized official documentation for full defense Sep
2027

14 Final defense and submission to the specialized scientific council
By Nov 2027

4. Conclusion

The results of this experimental study establish that chronic ulcerative inflammation of the large intestine triggers substantial, time-dependent degenerative modifications in distant musculoskeletal structures. The coxofemoral joint undergoes systematic structural breakdown characterized by a decrease in cartilage thickness, severe loss of functional chondrocytes due to suppressed Bcl-2 activity, chronic hyperplastic synovitis, and extensive subchondral bone resorption. These quantitative morphometric and immunohistochemical parameters provide crucial



baseline markers for the early prediction and potential pharmacological shielding of extraintestinal joint complications in clinical patients suffering from chronic IBD.

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