



MORPHOLOGICAL CHANGES OF COXOFEMORAL JOINT TISSUES IN A 60-DAY EXPERIMENTAL MODEL OF CHRONIC COLITIS

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Abstract: *Background: Comorbidities involving gastroenterological and musculoskeletal pathologies remain a significant challenge in modern medicine. Chronic inflammatory bowel diseases (IBD), such as ulcerative colitis and Crohn's disease, frequently provoke extraintestinal manifestations, prominently affecting large weight-bearing joints. This study aims to evaluate the comprehensive microscopic, morphological, and immunohistochemical shifts within the coxofemoral (hip) joint tissues—specifically the hyaline cartilage, subchondral bone, synovial membrane, and joint capsule—following a prolonged 60-day course of experimentally induced chronic acetic acid colitis in a rodent model.*

Methods: Two hundred and twenty adult male Wistar rats (weighing 180–220 g) were designated into a control group (n=100) and an experimental chronic colitis group (n=120) induced via serial rectal administration of a 3% acetic acid solution. Animals were systematically evaluated across progressive intervals, with a critical focus on the 60-day dynamic mark. Extracted joint blocks underwent specialized 10% buffered formalin fixation, 10% EDTA decalcification for 10–14 days, and subsequent paraffin embedding. Tissue thin-sections (4–5 mkm) were subjected to Hematoxylin and Eosin (H&E;) staining, Masson's trichrome



histochemical staining, and immunohistochemical (IHC) profiling using CD68, Ki-67, and Bcl-2 markers.

Results: By day 60 of chronic colitis, the coxofemoral joint demonstrated profound degenerative and destructive alterations. H&E; and Masson's trichrome stains highlighted a marked reduction in hyaline cartilage thickness, widespread chondrocyte lacunar emptying (necrosis), vertical disruption of isogenic groups, and structural fibrillation at the articular surface. The synovial membrane exhibited high-grade hyperplasia, accompanied by intense lymphocytic-neutrophilic sub-synovial infiltration, sustained microvascular hyperemia, and capillary stasis. Subchondral bone structures displayed extensive osteoporotic remodeling, marked by trabecular thinning, micro-fractures, vacant osteocyte lacunae, and a high concentration of active multi-nucleated osteoclasts in localized bone resorption zones. Masson's trichrome confirmed severe fibrotic acceleration and chaotic collagen alignment within the joint capsule. IHC data validated these pathologies through a severe increase in CD68(+) macrophage and osteoclast activity, an elevated synovial Ki-67 proliferative index contrasted by a dramatic collapse of chondrocyte Ki-67 expression, and a distinct suppression of the anti-apoptotic marker Bcl-2 within chondrocytes and osteocytes, reflecting accelerated programmed cell death mediated by systemic endotoxemia.

Conclusion: A 60-day course of chronic intestinal inflammation acts as a potent remote trigger for structural decay within the coxofemoral joint complex. The structural degradation mimics secondary osteoarthritis, driven by gut-barrier failure, systemic cytokine imbalance, microcirculatory breakdown, and accelerated cellular apoptosis. These findings establish vital morphometric benchmarks for early clinical prediction and prophylactic joint protection in patients suffering from chronic colitis.

Keywords: Chronic Colitis, Coxofemoral Joint, Hyaline Cartilage, Synovial Membrane, Subchondral Bone Remodeling, Immunohistochemistry, CD68, Ki-67, Bcl-2.



1. Introduction

The co-existence of gastroenterological and musculoskeletal system pathologies, widely classified under modern medical comorbidities, has evolved into an urgent problem in basic and clinical medical research. Chronic inflammatory bowel diseases (IBD), including classic archetypes like ulcerative colitis and Crohn's disease, represent systemic pathologies driven by dysregulated immune pathways. According to the World Health Organization (WHO) and global epidemiological data, the socioeconomic and healthcare burden of IBD is steadily expanding. Global epidemiological analyses indicate that in 2019, approximately 4.9 million individuals worldwide were afflicted with IBD, demonstrating a steep rise in incidence from 1990 through the mid-2020s. Large-scale meta-analyses covering 215 population-based studies revealed a global prevalence of

229.7 cases per 100,000 population, with an annual incidence rate of 9.7 cases per 100,000. For ulcerative colitis specifically, the prevalence stands at 120.4 cases, and the incidence is estimated at 5.0 cases per 100,000 individuals.

Importantly, the clinical course of chronic intestinal inflammation extends far beyond localized colonic tissue destruction. Prolonged mucosal inflammation induces systemic immune activation, causing widespread structural and functional defects in extraintestinal organs and tissues. WHO materials prominently include peripheral arthritis and axial spondyloarthropathies among the primary extraintestinal manifestations of Crohn's disease and chronic ulcerative colitis. Clinical statistics show that 30% to 40% of patients with long-standing chronic colitis develop secondary musculoskeletal complications.

Among these, pathologies affecting the coxofemoral (hip) joint are of paramount significance. The coxofemoral joint is the largest weight-bearing joint in the human body, responsible for translating core structural loads during locomotion. Secondary dystrophic, destructive, or osteoarthritis changes inside this joint drastically reduce physical capability and precipitate early patient disability. Pathogenetically, the breakdown of the intestinal mucosal barrier ("gut barrier")



facilitates systemic endotoxemia, allowing pro-inflammatory cytokines (such as TNF-alpha, IL-1beta, and IL-6) and bacterial lipopolysaccharides (LPS) into the systemic bloodstream. This triggers persistent microcirculatory stasis, microtromboid formation, localized hypoxia, and a severe imbalance in calcium-phosphorus metabolism within distant joint tissues.

However, despite these established clinical patterns, the precise, step-by-step ultra- and micromorphological and morphometric changes occurring inside the hyaline cartilage, subchondral bone, synovial membrane, and joint capsule over long periods of colitis remain insufficiently charted. Most current research focuses heavily on isolated biochemical markers without providing a quantitative, systemic structural map. Therefore, this study aims to systematically investigate and map the dynamic morphological, histochemical, and immunohistochemical transformations within the coxofemoral joint complexes using a standardized 60-day rodent model of chronic experimental colitis.

2. Materials and Methods

2.1. Experimental Animals and Model Design

The study utilized 220 healthy, sexually mature male Wistar rats weighing 180–220 grams. The animals were maintained under standard vivarium conditions with free access to water and balanced laboratory chow. All procedures adhered to international ethical standards for animal experimentation. The cohort was distributed into two primary arms:

1. Control Group (n=100): Comprising healthy animals receiving no inflammatory intervention.

2. Experimental Colitis Group (n=120): Subjected to the induction of chronic intestinal inflammation via the serial rectal administration of a 3% acetic acid solution to mimic the relapsing-chronic nature of human colitis. The experimental schedule was designed to track changes across multiple timeline milestones (14, 28, 45, 60, and 90 days), with this paper focusing heavily on the pivotal 60-day chronic phase.



2.2. Tissue Harvesting and Processing Logistics

At the designated 60-day milestone, animals were euthanized via dekapitatsiya under deep ether anesthesia. Macroscopic evaluation of the distal colon was immediately conducted to verify the severe presence of chronic inflammatory ulcers and mucosal thickening. Subsequently, the coxofemoral joint blocks were carefully excised bilaterally, ensuring the preservation of the joint capsule and adjacent periarticular soft tissues.

The structural density of the subchondral bone required an aggressive, step-by-step histological preparation protocol:

- **Fixation:** Joint blocks were immediately immersed in a 10% neutral buffered formalin solution for 24 to 48 hours to preserve cellular integrity.
- **Decalcification:** To soften the mineralized bone matrix prior to sectioning, tissues were submerged in a 10% ethylenediaminetetraacetic acid (EDTA) solution (pH 7.2–7.4) at room temperature for 10 to 14 days, monitored until a soft, uniform consistency was achieved.
- **Paraffin Embedding:** Tissues were dehydrated through a graded series of ethanol (70%, 80%, 90%, and 100%), cleared using xylene, and embedded into solid paraffin blocks.
- **Microtomy:** Using a high-precision rotary microtome, thin sections measuring 4–5 mkm were cut and mounted onto specialized slides.

2.3. Histological and Histochemical Staining

For general structural evaluation, sections were deparaffinized in xylene, rehydrated through descending alcohols (100% down to 70%), rinsed in distilled water, and stained with Hematoxylin and Eosin (H&E;). Under H&E;, cell nuclei stained deep blue-violet, while cytoplasm and collagenous connective tissues stained pinkish-red.

To specifically measure structural fibrosis, collagen deposition, and bone remodeling, Masson's Trichrome histochemical staining was executed. This technique yielded clear visual differentiation: collagen fibers stained intense bright



blue, cytoplasm and muscle elements stained red, and cell nuclei appeared dark brown-to-black. This method allowed for the precise calculation of the Fibrosis Index (%) and clearly demarcated new, unmineralized osteoid tissue (red-pink) from mature mineralized bone trabeculae (dark blue-violet).

2.4. Immunohistochemical (IHC) Profiling

To reveal the molecular kinetics driving the tissue degradation, sections were mounted on kation-coated slides (poly-L-lysine) and processed for IHC using a panel of three specific monoclonal antibodies:

1. CD68 (Macrophage Marker): Employed to map the density and distribution of infiltrating macrophages in the synovial membrane and multinucleated osteoclasts in subchondral bone resorption zones. Cytoplasmic/membranous expression presented as a distinct brown stain.

2. Ki-67 (Proliferation Marker): Used to gauge the active mitotic/proliferative capacity of chondrocytes and synoviocytes, localizing exclusively within cell nuclei (brown).

3. Bcl-2 (Anti-Apoptotic Marker): Used to assess cell survival capability within the cartilage matrix and subchondral osteocytes, localizing within the cytoplasm and mitochondrial membranes (brown).

Quantitative digital morphometry was performed using specialized imaging software to record cartilage thickness, trabecular indices, cell densities, and staining intensities.

3. Results and Discussion

3.1. Hyaline Cartilage Degradation

By day 60 of experimental chronic colitis, the coxofemoral hyaline cartilage exhibited severe degenerative and osteoarthritis degradation. Structural evaluation via H&E; and Masson's trichrome staining showed a substantial, non-uniform decrease in total cartilage thickness compared to the uniform, healthy layers observed in the control group. The smooth superficial layer of the articular cartilage was replaced by distinct surface defects, vertical cracks, and advanced fibrillation zones.



At the cellular level, there was a profound disruption in the alignment of isogenic cell groups. The standard columnar arrangement of chondrocytes was completely disorganized. Widespread chondrocyte necrosis was evident, characterized by extensive clusters of empty chondrocyte lacunae. Masson's trichrome staining demonstrated a localized drop in blue color intensity across the cartilage matrix, indicating severe depletion of type II collagen and proteoglycan ground substance.

IHC analysis provided key mechanistic insights into this cartilage loss. The expression of the anti-apoptotic protein Bcl-2 within chondrocytes fell drastically at day 60, confirming that systemic inflammatory endotoxemia and high levels of circulating cytokines (TNF-alpha, IL-1beta) trigger rapid chondrocyte apoptosis. Furthermore, Ki-67 nuclear expression within the cartilage was highly suppressed. While early phases of colitis (days 14–28) often show temporary compensatory chondrocyte clustering, the 60-day mark revealed a complete failure of cartilage regeneration.

3.2. Synovial Membrane Hyperplasia and Capsular Fibrosis

The synovial membrane responded to chronic intestinal inflammation with severe, persistent synovitis. Microscopic analysis showed marked synoviocyte layer hyperplasia, with the lining expanding from its typical thin layer to a thick, multi-row layout. The sub-synovial connective tissue space was heavily infiltrated by a dense mix of lymphocytes, plasma cells, and neutrophils, demonstrating a profound immune response.

Severe microvascular changes were observed throughout the synovial stromal bed. Capillaries and venules showed significant dilation (hyperemia) and widespread red blood cell stasis. IHC evaluation marked a severe influx of CD68(+) macrophages within both the synovial lining and sub-synovial spaces, indicating high-grade chronic activation. Synovial Ki-67 staining revealed a high proliferative index among synoviocytes, corresponding to the formation of aggressive, hyperplastic, pannus-like tissue capable of invading the adjacent joint margins.



Simultaneously, the joint capsule underwent extensive remodeling. Masson's trichrome staining showed an accelerated accumulation of dense, bright blue collagen fiber bundles. These collagen bundles were arranged chaotically, accompanied by severe edema and fibroblastic proliferation. Digital morphometry confirmed a dramatic spike in the Fibrosis Index (%) within the capsule, explaining the joint stiffness and restricted mobility frequently observed in long-standing clinical colitis.

3.3. Subchondral Bone Remodeling and Osteoporosis

The subchondral bone plate and underlying trabecular bone experienced intense pathological remodeling by day 60. The orderly architectural framework of healthy bone trabeculae was replaced by a highly disorganized osteoporotic pattern. Histological sections showed severe thinning and frequent fragmentation of the bony trabeculae.

A defining feature of the 60-day chronic phase was the high presence of empty osteocyte lacunae within the

trabeculae, indicating widespread osteocyte death. Concurrently, active bone resorption zones were highly prominent along the trabecular surfaces. These resorption pits contained a large number of large, multi-nucleated CD68(+) osteoclasts. This osteoclast proliferation confirms that chronic systemic colitis speeds up bone reabsorption, outpacing bone formation and culminating in subchondral osteoporosis.

Masson's trichrome staining clearly differentiated these dynamics. It revealed patches of unmineralized, pale red-pink osteoid tissue surrounding fragments of mature, dark blue mineralized bone. This structural disconnect highlights a significant impairment in normal bone mineralization, directly tied to the systemic disruption of calcium-phosphorus metabolism caused by chronic gut barrier failure.

Joint Tissue Component	Primary Morphological Observations (Day 60)
Quantitative Markers	Key Molecular/IHC Findings



Hyaline Cartilage Surface fibrillation, vertical cracks, disorganized isogenic groups, extensive chondrocyte necrosis. Decreased thickness; low blue intensity (Masson's Trichrome) showing matrix depletion. Widespread suppression of Bcl-2 (high apoptosis); minimal Ki-67 expression (exhausted regeneration).

Synovial Membrane Multi-row synoviocyte hyperplasia, dense lymphocytic-neutrophilic infiltration, vascular hyperemia/stasis. Marked increase in total membrane thickness and microvessel density. High accumulation of CD68(+) macrophages; elevated Ki-67 index in synoviocytes.

Subchondral Bone Severe trabecular thinning, micro-fractures, extensive vacant osteocyte lacunae, active osteoporotic resorption. Decreased trabecular density; prominent unmineralized osteoid zones. High concentrations of CD68(+) multi-nucleated osteoclasts in Howell's lacunae.

Joint Capsule Chaotic collagen bundle arrangement, severe interstitial edema, advanced tissue sclerosis. High Fibrosis Index (%) via dense collagen staining (Masson's Trichrome). Fibroblastic proliferation driven by systemic pro-inflammatory cytokine pathways.

3.4. Pathogenetic Integration

The morphological changes observed at day 60 highlight a distinct "gut-joint axis" pathway. Long-standing intestinal inflammation breaks down the protective epithelial barrier, allowing lipopolysaccharides and luminal antigens into the bloodstream. This causes a persistent elevation of systemic pro-inflammatory cytokines like TNF-alpha and IL-1beta. When these inflammatory mediators reach the highly vascularized synovial membrane and subchondral bone of the coxofemoral joint, they cause microvascular stasis and localized hypoxia. This chronic hypoxic environment suppresses Bcl-2 protection, triggering widespread apoptosis in both chondrocytes and osteocytes. Concurrently, it stimulates the overproduction of RANKL, driving the proliferation of CD68(+) osteoclasts that rapidly break down the subchondral bone. This combined loss of cellular support and



matrix integrity transforms a localized intestinal disease into a destructive joint pathology, accurately mirroring the secondary osteoarthritis seen in human IBD patients.

4. Conclusion

This study provides a detailed, quantitative analysis of the severe extraintestinal damage inflicted on the coxofemoral joint by a prolonged 60-day course of experimental chronic colitis. The data demonstrate that chronic intestinal inflammation triggers widespread, destructive changes across all components of the joint complex, including the hyaline cartilage, synovial membrane, subchondral bone, and joint capsule. These changes are characterized by accelerated chondrocyte apoptosis, severe hyperplastic synovitis, extensive capsular fibrosis, and aggressive osteoclastic bone resorption. Characterizing these clear morphological and immunohistochemical patterns provides valuable diagnostic criteria. These tissue benchmarks can help clinicians predict joint complications early and develop targeted, proactive therapies to protect the musculoskeletal health of patients suffering from chronic inflammatory bowel diseases.

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