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Hip osteoarthritis – histopathological aspects

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Abstract

Osteoarthritis is a complex, degenerative disease that can affect all the anatomical structures of the synovial joints. Most frequently, the joints of the hand, hip, and knee are affected, especially in the elderly patients. In our study, we evaluated 27 femoral heads, examining the histopathological changes that occurred in the articular cartilage, subchondral bone, and perisynovial soft tissues. At the level of the articular cartilage, there were observed a reduction in thickness, deformation of the articular surface, degradation of the cartilaginous matrix, the occurrence of fissures or fractures in the cartilaginous piece, a reduction in the number of chondrocytes, and changes in their morphology. In the subchondral bone, a rarefaction of the bone trabeculae and a reduction in their thickness were observed, along with an increase in the size of the alveolar cavities. These changes were accompanied by the formation of cystic cavities, non-homogeneous hypertrophy of the subchondral bone plate as a response to the reduction in thickness and change of the articular cartilage structure, or the reduction in the thickness of the subchondral bone plate. The trabecular bone exhibited an atrophic endosteum, absence of bone remodeling processes, cracks or even fractures in the trabecular bone. Likewise, we observed rare ectopic osteogenesis processes, either endochondral or desmal ones, forming osteophytes. The synovium and perisynovial connective tissue contained immune cells, vascular endothelial cells, fibroblasts, adipocytes, and other mesenchymal-derived stromal cells. The immunohistochemical study highlighted the presence of T-lymphocytes, B-lymphocytes, and macrophages, cells capable of synthesizing and releasing matrix metalloproteinases that are involved in the degradation of the articular cartilage. Exploration of cell proliferative capacity using the proliferating cell nuclear antigen (PCNA) showed that, in the articular cartilage, there are few cells (chondrocytes) capable of proliferation, while in the synovium there are numerous young fibroblasts capable of mitotic division.

Keywords: hip osteoarthritis, articular cartilage, bone, chondrocytes, synovium, inflammation.

Introduction

Osteoarthritis (OA) is a degenerative disease of the mobile (synovial) joints that affects up to 70% of the elderly population. All diarthroses can be affected by OA, but the joints most frequently affected are the hand, hip, and knee. According to studies on individuals who live up to 85 years, there is an estimated 25% risk of developing symptomatic hip OA [1]. Globally, over 300 million people suffer from OA [2]. Pathologically, OA is characterized by complex lesions such as: deterioration of articular cartilage, changes in subchondral bone, formation of osteophytes, muscular changes, and inflammation of the synovial tissue [3]. The pathogenesis of hip OA is incompletely understood. All studies suggest that pathological biomechanical stress is the problem that disrupts the homeostatic balance between the synthesis and degradation of articular tissues. Biomechanical stress is generated by certain risk factors that act both at the joint level and at the individual level and play a central role in the development and evolution of OA. Here we can include mechanical factors (obesity and excessive physical work, trauma), dysplastic lesions, vascular changes, ventilatory disorders, advanced age, smoking, etc. [4–6]. Synovial joints have a more complex structure, being composed of articular cartilage, subchondral bone, and a joint capsule, which is composed on the inside with synovial membrane and reinforced on the outside by ligaments. Diseases that affect one of the components of a joint will eventually lead to secondary changes in the other components, developing joint insufficiency that causes pain and duces joint movements for the patient [7–9]. OA is one of the most common and significant causes of chronic pain and disability. It severely affects the quality of life of patients and increases the socioeconomic burden [10–12]. Globally, hip and knee OA have been ranked as diseases with significant contributions to the increase in global disabilities [13, 14]. The prevalence of OA increases with age; therefore, the most common and severe cases are found in elderly individuals [15].

Aim

In the present study, we aimed at assessing the histopathological (HP) changes occurring in hip OA.

Materials and Methods

The studied material consisted of 27 femoral heads obtained from patients clinically and paraclinically diagnosed with hip OA, hospitalized in the Department of Orthopedics, Emergency County Hospital, Drobeta-Turnu Severin, Romania, between 2014–2019, who required total hip arthroplasty. From every patient, we obtained an informed consent which comprised all the information regarding the disease, the surgical intervention, and the subsequent use of the biological material collected during the arthroplasty operation for scientific study, as well as the handling of private data.

The biological material was placed in a 10% neutral buffered formalin fixative solution for 10–15 days at room temperature in airtight glass containers, after which it was initially cut into thin longitudinal sections of approximately 1–1.5 mm thickness using a small saw equipped with a fine blade and a vice to hold and secure the femoral head or neck. The main objectives of fixation were stopping vital processes in cells and tissues, preventing structural changes due to bacterial attack, and preparing cells and tissues for the appropriate staining procedure [16]. The bone fragments thus processed were placed in a decalcifying solution of 7% trichloroacetic acid, on the plate of a magnetic stirrer to accelerate the loss of calcium salts from the bone tissue structure' through movement, and to transform the bone tissue into a soft connective tissue that could be embedded in paraffin. The decalcifying solution was changed every three days for a faster and more uniform decalcification. After completing the decalcification, the biological material was washed with tap water for about 12 hours, after which it was embedded in paraffin according to the classical HP protocol. Then, serial sections were made using the Microm HM350 rotary microtome, equipped with a special section transfer system on water bath (Section Transfer System, STS), a high-precision device that allowed us to create high-quality histological cups with uniform thickness to prevent microscopic artifacts.

Finally, the histological sections were stained using the Hematoxylin–Eosin (HE) method.

For immunohistochemistry (IHC) studies, the histological sections were placed on slides coated with poly-L-lysine.

The following antibodies were used: anti-proliferating cell nuclear antigen (PCNA) (monoclonal mouse anti-PCNA, PC10 clone, 1/100 dilution, Dako) for highlighting cells with proliferative potential; anti-cluster of differentiation (CD)45RO (monoclonal mouse anti-human CD45RO, UCHL1 clone, 1/50 dilution, Dako) for the detection of native memory T-lymphocytes; anti-CD3 (monoclonal mouse anti-human CD3, F7.2.38 clone, 1/25 dilution, Dako) for the detection of T-cells; anti-CD20 (monoclonal mouse anti-human CD20cy, L26 clone, 1/50 dilution, Dako) for the detection of B-cells; anti-CD68 (monoclonal anti-human CD68, KP1 clone, 1/100 dilution, Dako) for the detection of macrophages; anti-matrix metalloproteinase (MMP)1 (monoclonal mouse anti-human tissue inhibitor of metalloproteinase 1, VT7 clone, 1/50 dilution, Dako) for highlighting cells that produce MMP1; anti-MMP9 (polyclonal rabbit anti-human MMP9, 1/150 dilution, Dako) for highlighting cells that produce MMP9; anti-MMP13 (anti-MMP-13 VIIIA2, NB110-5919 clone, 1/150 dilution, Novus) for highlighting cells that produce MMP13.

Results

Changes in the articular cartilage

Articular cartilage is the most affected structure in OA. Radiologically, as we also mentioned in previous studies, in the early stages of the disease, we may observe a reduction in the joint space at the level of the affected coxofemoral joint. In reality, these radiological aspects represent the image of the reduction in the thickness of the articular cartilages, which, being transparent, in contrast with the underlying bone tissue, give the aspect of an empty space.

We must mention that the cartilaginous tissue covering the femoral head, which we studied in terms of microscopic changes, is a hyaline cartilage with certain characteristics: (*i*) it is not covered by the perichondrium, its nourishment being provided by the synovial fluid; (*ii*) it presents three structurally different zones – the superficial zone, the middle zone and the deep zone.

The articular cartilage is a semi-rigid, elastic tissue that has a smooth and lubricated surface, necessary for the movement of the bones in the joint; it plays a major role in the absorption and dissipation of mechanical forces to the underlying subchondral bone.

The microscopic aspects obtained by us have shown multiple changes in the articular cartilage, varying from one patient to another depending on the evolutionary stage of the condition and even from one area to another within the same articular cartilage.

In the early stages of the disease (grade II OA), in the deep zone of the cartilage, we identified the presence of well-defined chondrocyte clusters, distinct from the rest of the extracellular matrix (ECM), bordered by a zone of dense, a basophilic cartilage matrix, microscopic aspects that approach a normal state of cartilage. Inside the chondrocytic globules, the isogenous series of small chondrocytes with small, acidophilic nuclei and reduced cytoplasm were highlighted, indicating cellular distress (Figure 1). In the middle zone of the cartilage, the chondrocyte lacunae were more difficult to identify due to the fact that the connective matrix had a changed aspect, becoming acidophilic, similar to the connective matrix between the chondrocyte lacunae, as a result of the biochemical changes in the cartilaginous matrix (Figure 2). In the superficial zone of the cartilage, the absence of chondrocytes was observed, the number of chondrocytes was reduced compared to the underlying areas, and the series of isogenic chondrocytes were quite rare. Chondrocytes appeared smaller in size, with reduced cytoplasm. Empty chondrocytes resulting from the death of superficial chondrocytes were frequently observed. The surface of the articular cartilage was often irregular and rough (Figure 3).

In the advanced stages of the disease (stages III or IV OA), the lesions of the articular cartilage were more extensive. Cartilage cracks were identified (Figures 4 and 5), deformation of the articular surface, degradation of the cartilage matrix, reduction in the number of chondrocytes, and changes in their morphology (Figures 6 and 7). In the case of severe forms of OA, clinically manifested by intense pain and functional impairment up to ankylosis, areas of old cartilaginous fractures were sometimes identified, associated with the formation of granulation connective tissue with reparative characteristics (Figures 8–11).

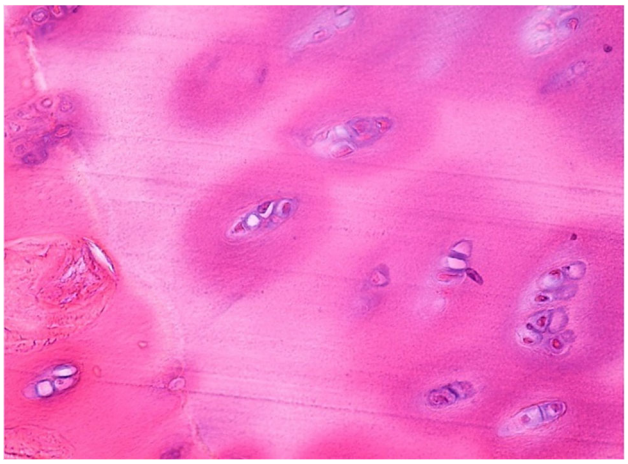

Figure 1 – ***Image of articular cartilage from the deep zone of a patient with grade II osteoarthritis, showing the presence of chondrocyte globules and relatively well-defined interstitial fundamental matrix. HE staining, ×200. HE: Hematoxylin–Eosin.***

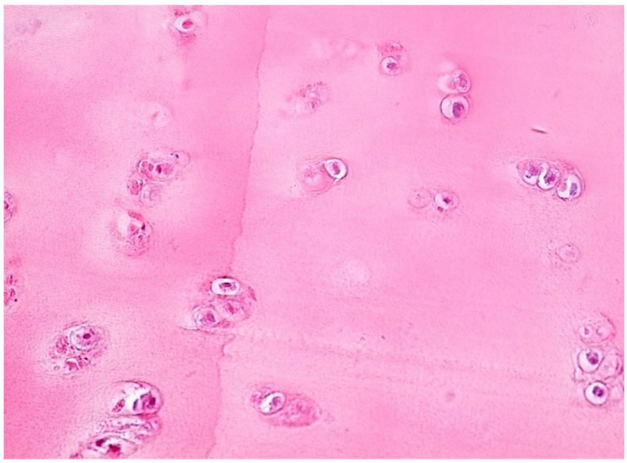

Figure 2 – ***Image of articular cartilage from the middle zone where a reduction in the number of chondrocytes and chondrocytic globules is noted. HE staining, ×200.***

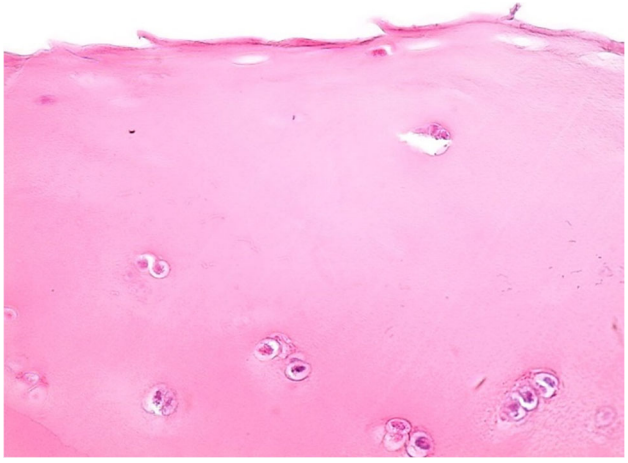

Figure 3 – ***Image of articular cartilage from the superficial zone where the absence of chondrocyte clusters, the reduction in the number of isogenous groups, the reduction in the number of chondrocytes, the increase in the volume of the cartilage matrix, and the alteration of the articular surface are noted. HE staining, ×200.***

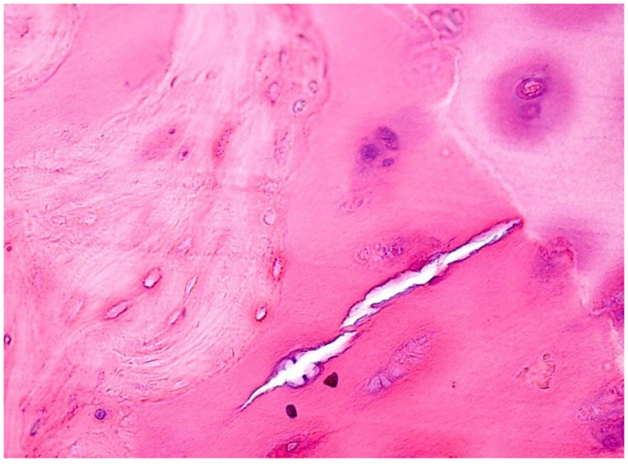

Figure 4 – ***Articular cartilage with a non-homogeneous structure, with varied staining and deep fissures as a consequence of biochemical changes in the cartilage matrix. HE staining, ×200.***

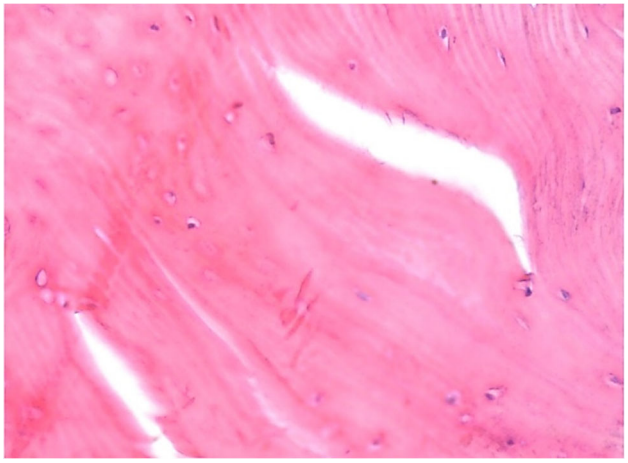

Figure 5 – ***Multiple fissures in the middle zone of the articular cartilage. HE staining, ×200.***

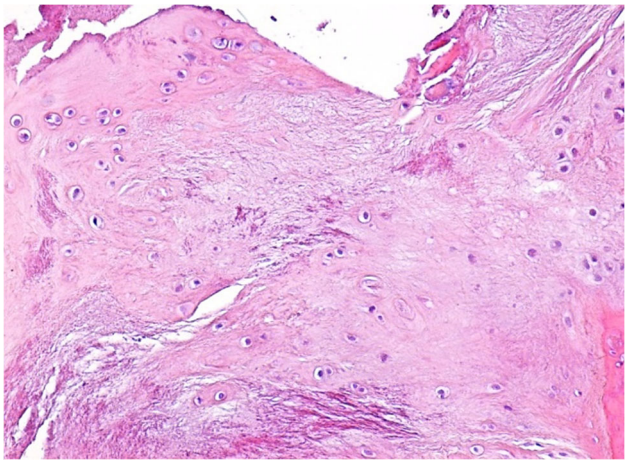

Figure 6 – ***Articular cartilage with a non-homogeneous structure, due to the reduction and modification of the cartilage matrix, with the heterogenous distribution of chondrocytes, a more intense highlighting of the fibrillar structure, and an irregular articular surface. HE staining, ×200.***

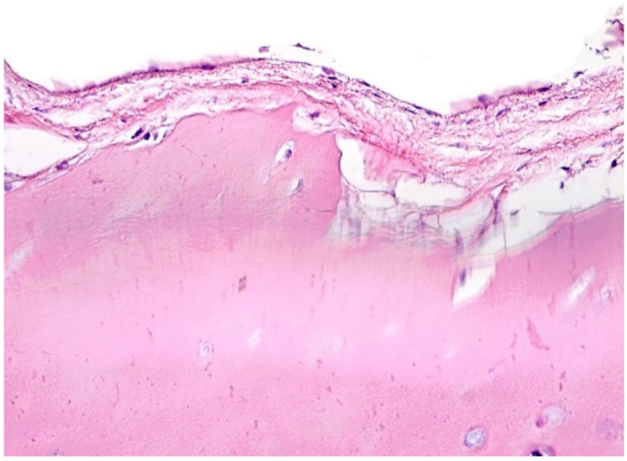

Figure 7 – ***Microscopic image of the superficial zone of the articular cartilage from a case of stage IV hip osteoarthritis, where a marked reduction of chondrocytes, alteration of the cartilage matrix with chondrocyte necrosis, and highlighting of the fibrillar structure of the cartilage are observed. HE staining, ×200.***

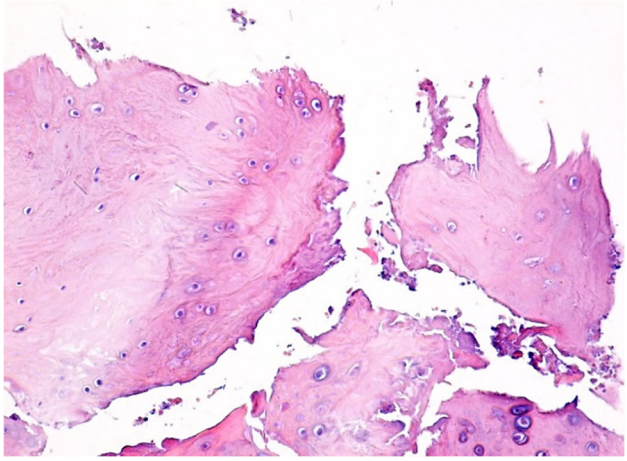

Figure 8 – ***Multiple fracture lines with areas of necrosis at the level of the articular cartilage. HE staining, ×200.***

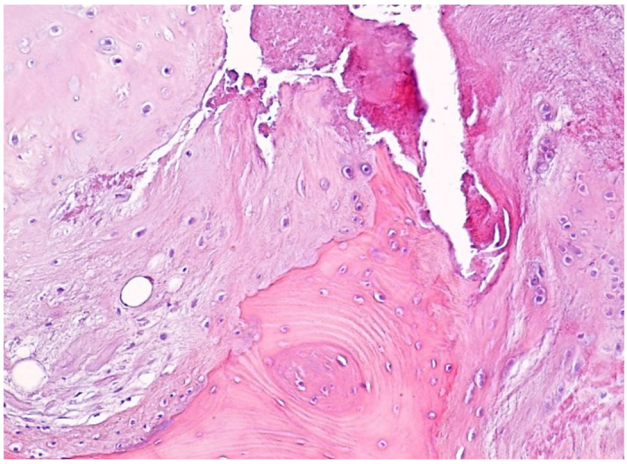

Figure 9 – ***Deep fracture of the articular cartilage down to the level of the subchondral bone. HE staining, ×200.***

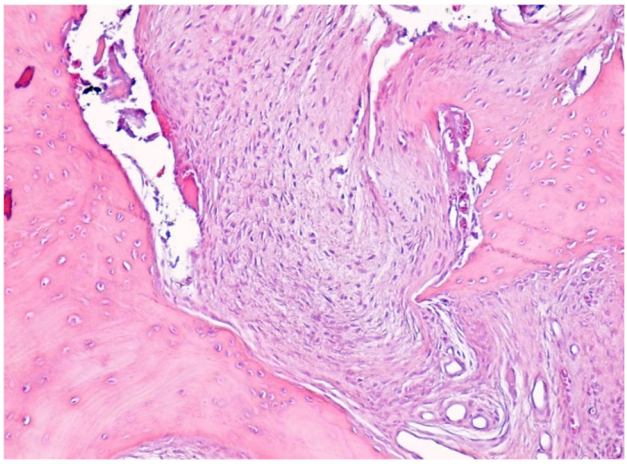

Figure 10 – ***Image of young granulation tissue, rich in fibroblasts, developing in the old fracture area of the articular cartilage. HE staining, ×200.***

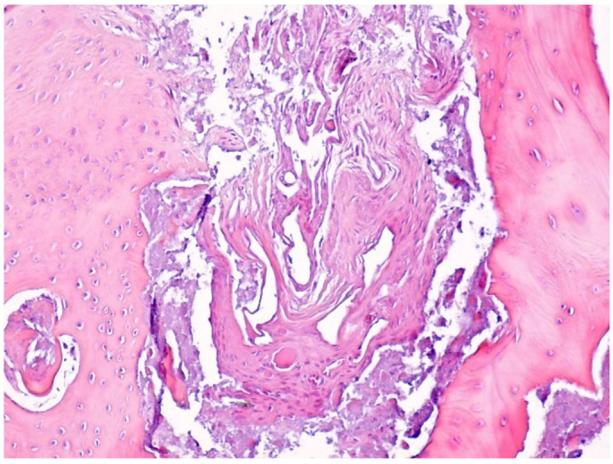

Figure 11 – ***Mature granulation tissue with a scar-like appearance, rich in collagen fibers, developed in an area of old joint cartilage fracture. HE staining, ×200.***

Changes in the subchondral bone

The articular cartilage is tightly attached to the subchondral bone, forming a functional unit that responds in a coordinated manner to the mechanical demands placed on the joint. For this reason, lesions of the articular cartilage are accompanied by changes in the bone of the femoral head. The macroscopic examination of the surgical excision pieces showed, depending on the progression stage of the disease, deformities of the femoral head, presence of osteophytes, or an irregular appearance of the articular surface.

We must mention that at the level of the subchondral bone, there are two bony structures that are affected in OA: the subchondral bone plate and the trabecular bone.

The microscopic examination performed on decalcified bone fragments revealed multiple changes in the bone architecture. The overall examination with small microscopic objectives of the spongy bone of the femoral head showed, in most cases, a thinning of the bony trabeculae and a reduction in their thickness, along with an increase in the size of the alveolar cavities and even the formation of cystic cavities. The myeloid tissue was replaced by unilocular adipose tissue. These changes indicate a significant reduction in the mechanical strength of the bone and explain the deformation of the femoral head.

The examination of HP preparations with high-power microscopic objectives showed in some cases a non-homogeneous hypertrophy of the subchondral bone plate as a response to the reduction in thickness and change of the articular cartilage structure (Figure 12).

In other cases, we observed a reduction in the thickness of the subchondral bone plate (Figure 13). The examination of the subchondral trabecular bone tissue showed a reduction in the thickness of the bone trabeculae, endosteal atrophy, absence of bone remodeling processes, fissures, and even fractures of the trabeculae (Figures 14 and 15). Quite rarely, at the edge of the articular cartilage, ectopic osteogenesis processes of the endochondral or desmal type (Figures 16 and 17) were identified, which underlie the development of osteophytes.

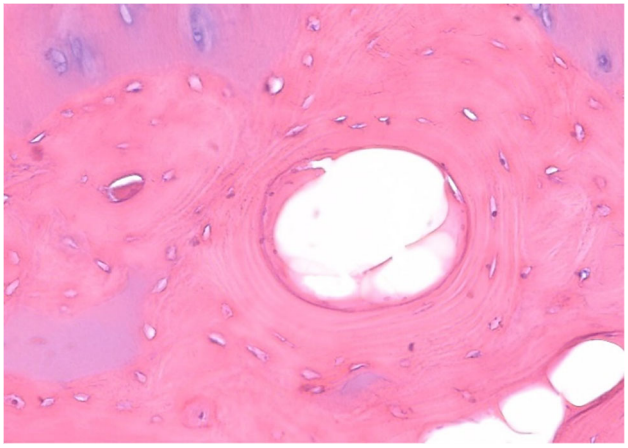

Figure 12 – ***Thickened basal plate due to the formation of new haversian-type bone tissue progressing towards the deep part of the articular cartilage, in a patient with grade III osteoarthritis. HE staining, ×200.***

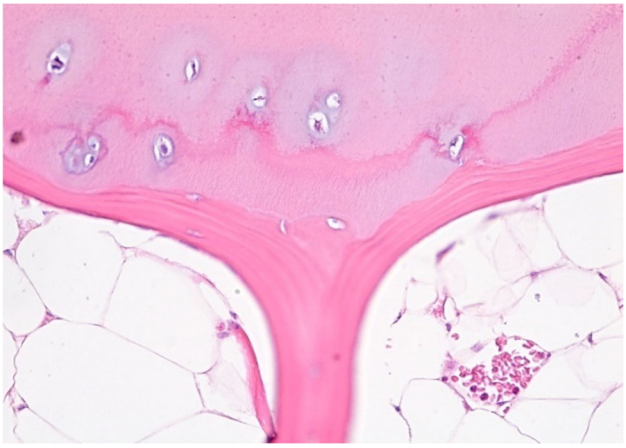

Figure 13 – ***Basal bone plate formed by several (3–5) bone lamellae with the aspect of a spongy bone. HE staining, ×200.***

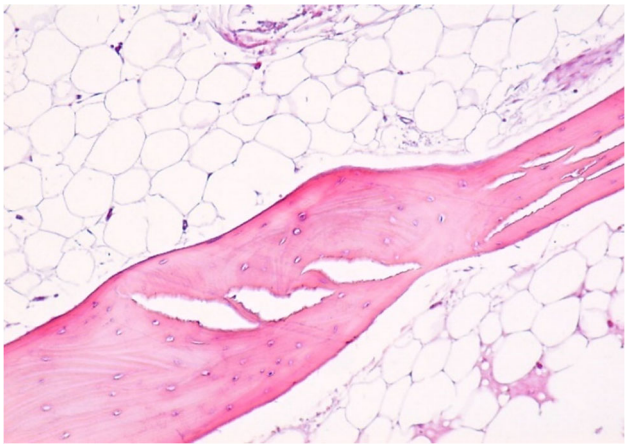

Figure 14 – ***Subchondral bone trabecula at the level of the femoral head, with multiple bone fissures in its structure. HE staining, ×200.***

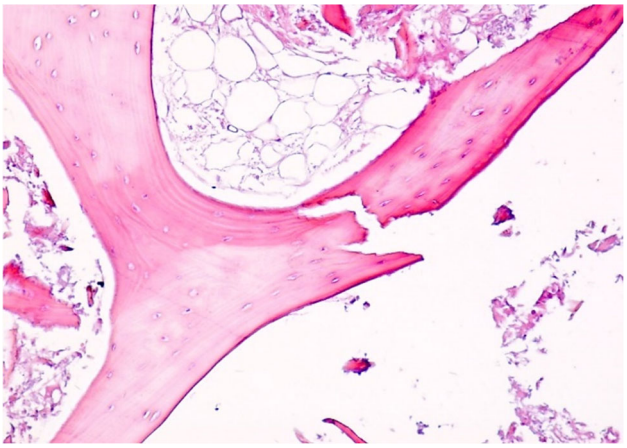

Figure 15 – ***Fractured subchondral bone trabecula. HE staining, ×200.***

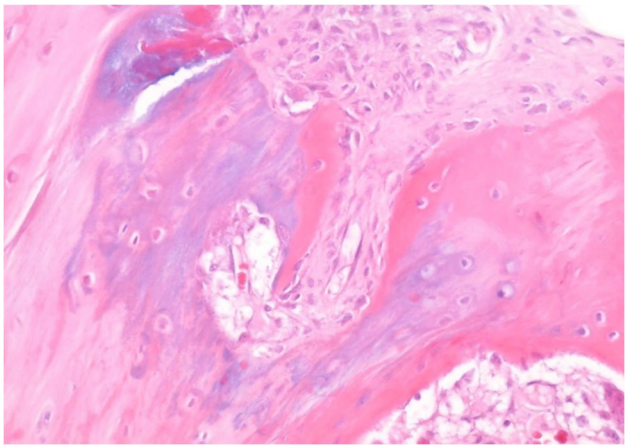

Figure 16 – ***Ectopic endochondral ossification at the margin of the articular cartilage, with the formation of osteophytes. HE staining, ×200.***

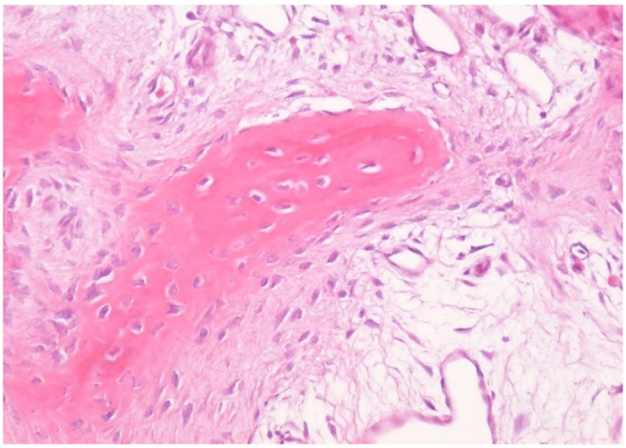

Figure 17 – ***Ectopic desmal ossification, with the formation of osteophytes. HE staining, ×200.***

Synovial changes

In our study, by the term "synovium" we did not refer only to that microscopic synovial membrane that lines the inner surface of the articular capsule, but we also analyzed the inner third of the articular capsule, due to the morphological and functional interpenetration of these structures.

Thus, we found that synovium is a heterogeneous connective tissue that contains immune cells, vascular endothelial cells, fibroblasts, adipocytes, and possibly other mesenchymal stromal cells. Having this structure, we consider the synovium to be the main regulator of intra-articular inflammation in OA, being highly vascularized and containing immune cells (Figure 18).

The study of the inflammatory infiltrate, using IHC techniques, selectively highlighted the types of inflammatory cells present in the synovium. T- and B-lymphocytes, and macrophages were identified (Figures 19–22), cells capable of synthesizing and releasing MMPs that are involved in the degradation of the articular cartilage (Figures 23–25).

Exploration of the cell multiplication capacity using PCNA showed that at the level of the articular cartilage, there are few cells (chondrocytes) capable of multiplication, while in synovium, there are numerous young fibroblasts capable of mitotic division (Figures 26 and 27).

Discussions

Experts, researchers, and doctors gathered within the *International Society for Osteoarthritis Research* and defined OA as a chronic disease of mobile joints, characterized by the progressive change of articular cartilage, subchondral bone, and chronic inflammation of the soft tissues of the joint [17–19].

The coxofemoral joint is one of the most frequently affected sites of OA, a disease that affects over 240 million people worldwide [20–22]. As the population ages, the prevalence of OA increases and is associated with rising medical and social costs [23, 24]. The articular cartilage is the first and most affected joint structure in OA, as the cartilage tissue has a very weak repair and regeneration capacity, despite containing a considerable number of progenitor cells [25–27]. Likewise, the structure of articular cartilage is slightly different from other types of cartilage in that it has a smaller number of chondrocytes spread across a large amount of ECM. Additionally, in OA, cartilage metabolism is dominated by catabolic processes that lead to the breakdown and loss of tissue components. At the onset of the disease, there is a loss of proteoglycans (aggrecan), followed by the deterioration of the collagen network [28].

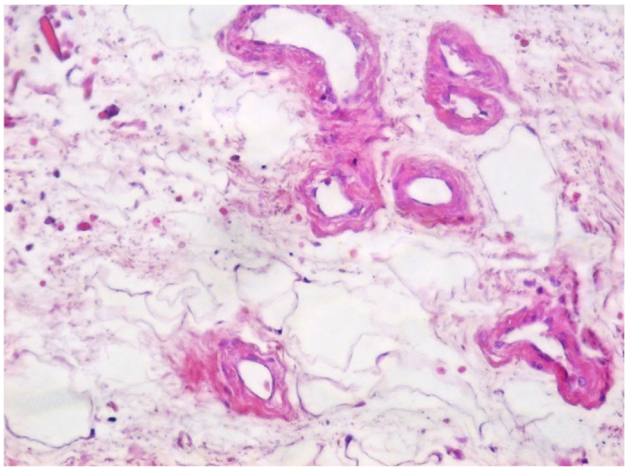

Figure 18 – ***Loose connective tissue in the synovium, containing numerous blood vessels and a moderate inflammatory infiltrate. HE staining, ×100.***

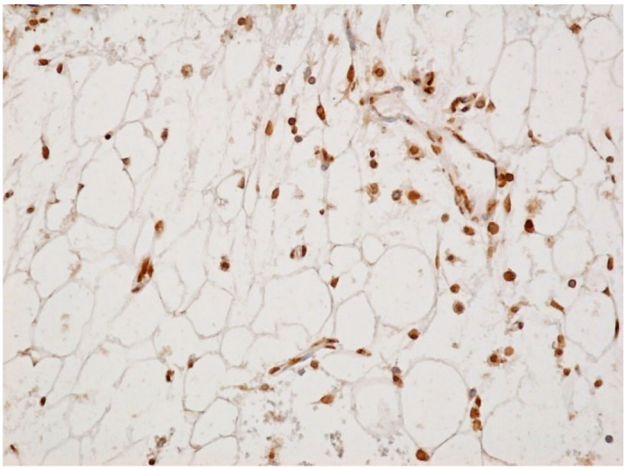

Figure 19 – ***Diffuse inflammatory infiltrate, rich in lymphocytes. Immunostaining with anti-CD45RO antibody, ×200. CD45RO: Cluster of differentiation 45RO.***

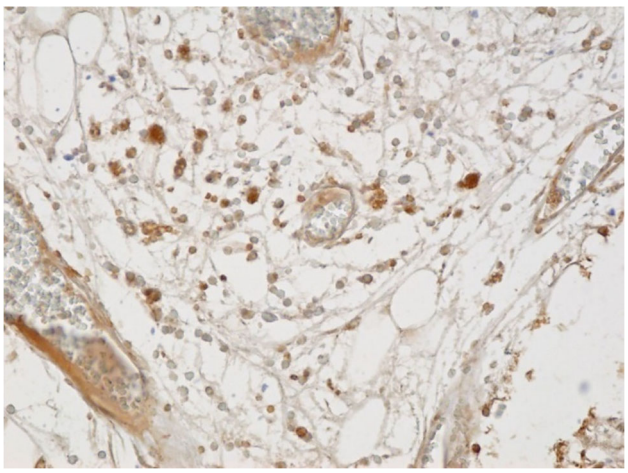

Figure 20 – ***Diffusely scattered CD3+ T-lymphocytes in the synovium. Immunostaining with anti-CD3 antibody, ×200. CD3: Cluster of differentiation 3.***

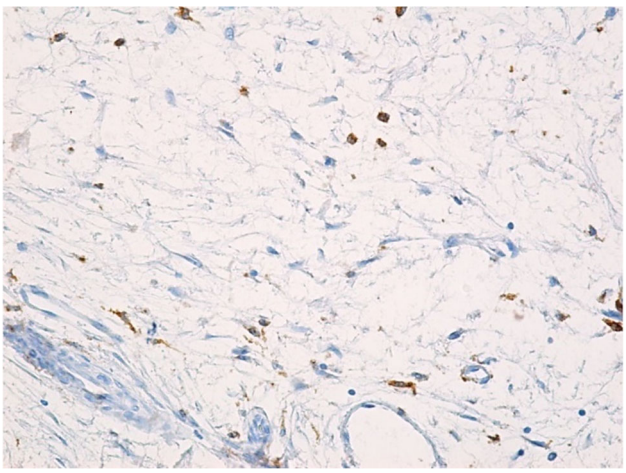

Figure 21 – ***Rare B-lymphocytes in the synovial membrane. Immunostaining with anti-CD20 antibody, ×200. CD20: Cluster of differentiation 20.***

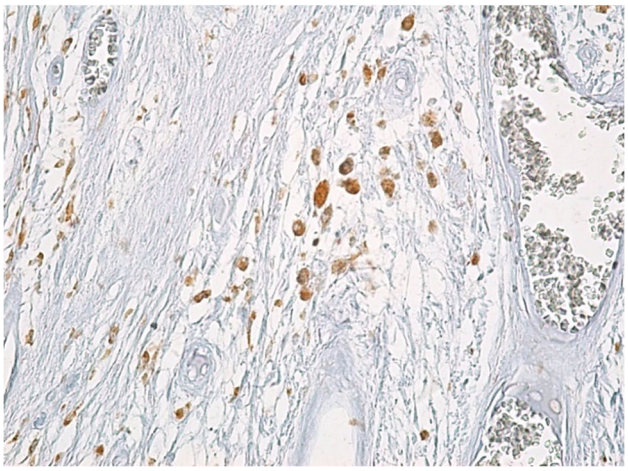

Figure 22 – ***Chronic inflammatory infiltrate predominantly composed of macrophages. Immunostaining with anti-CD68 antibody, ×200. CD68: Cluster of differentiation 68.***

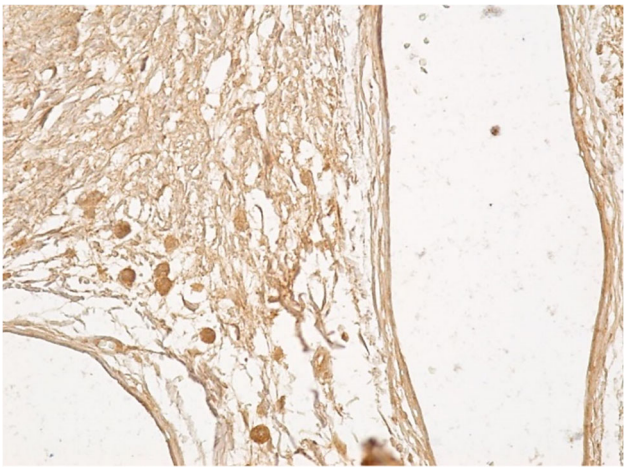

Figure 23 – ***Inflammatory-type cells (macrophages) capable of synthesizing and secreting MMP1. Immunostaining with anti-MMP1 antibody, ×200. MMP1: Matrix metallo-proteinase 1.***

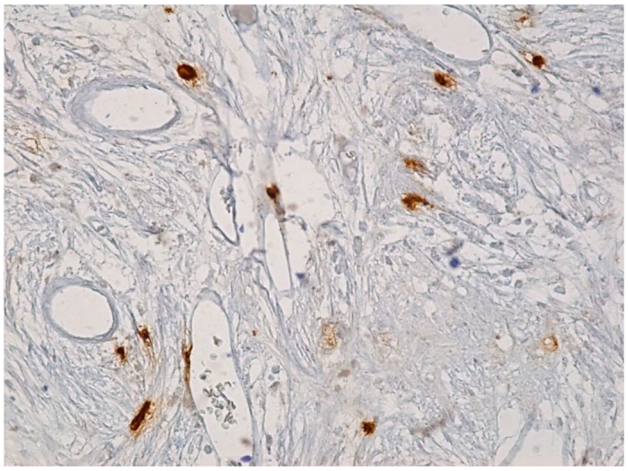

Figure 24 – ***Inflammatory-type cells (macrophages) capable of synthesizing and secreting MMP9. Immunostaining with anti-MMP9 antibody, ×200. MMP9: Matrix metallo-proteinase 9.***

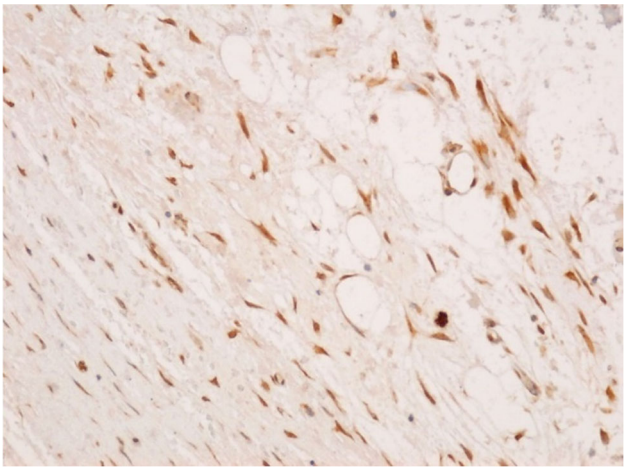

Figure 25 – ***Inflammatory-type cells and fibroblasts present in the synovium, producing MMP13. Immunostaining with anti-MMP13 antibody, ×200. MMP13: Matrix metallo-proteinase 13.***

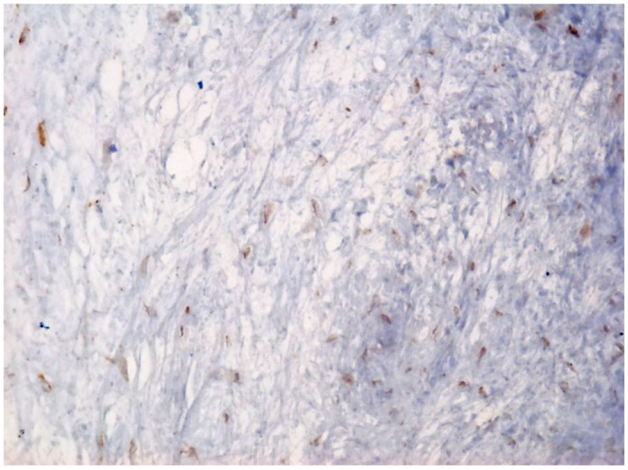

Figure 26 – ***Articular cartilage with the cartilage matrix destroyed, with few chondrocytes that retained their ability to multiply. Immunostaining with anti-PCNA antibody, ×200. PCNA: Proliferating cell nuclear antigen.***

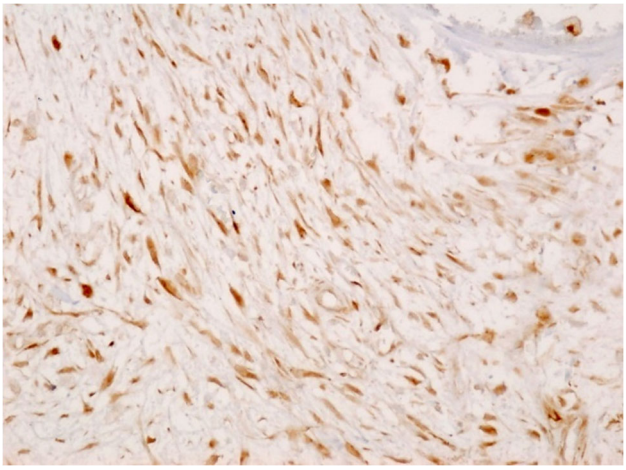

Figure 27 – ***Synovium with multiple fibroblast-like cells with high proliferative capacity. Immunostaining with anti-PCNA antibody, ×200.***

Most studies show that OA begins long before the onset of the first clinical signs, through cellular and molecular changes that progress slowly. These HP changes manifest, as we also showed, through the change of the microscopic aspect of the cartilage matrix.

Articular cartilage is composed of cells (chondrocytes)

and a cartilage matrix, which is made up of proteoglycans, glycosaminoglycans, and type II collagen. The microscopic architecture and functions of cartilage are maintained through a normal metabolic and synthetic activity of chondrocytes [29]. In OA, under the influence of etiopathogenic factors (mechanical, metabolic, genetic), the metabolism of chondrocytes is altered so type I and III collagen are synthesized, enzymes and inflammatory mediators are released. The cartilage matrix is altered, leading to the progressive deterioration of articular cartilage and the compromise of joint function [30, 31]. We believe that repetitive mechanical stress, induced by etiopathogenic factors, leads to a reduction in the quantity and quality of proteoglycans and collagen in the ECM, increased release of proinflammatory mediators, and cellular OA can be primary (idiopathic) caused by overuse and wear of the articular cartilage, and secondary, caused by trauma, dysplastic lesions, inflammatory or infectious processes, etc. [32, 33]. Regardless of the form, the clinical symptoms and HP changes are similar. These changes are found in the cartilage, bones, synovium, ligaments, and periarticular adipose tissues [34].

In our study, in addition to cellular and cartilage matrix changes, we identified the presence of microcracks in the cartilage structure that partially or completely affected the thickness of the articular cartilage. Some studies show that these microcracks facilitate increased vascularization and the bidirectional passage of cytokines and growth factors through the osteochondral junction, thus connecting the cartilage and subchondral bone biochemically and mechanically [35–37]. The progressive degeneration of articular cartilage indicates that the normal balance of chondrocyte metabolic activities was severely disrupted. It is believed that stressed articular cartilage releases pro-inflammatory cytokines and osteoclast-stimulating molecules that reach the subchondral bone to modify subchondral bone remodeling [38]. The proinflammatory signaling molecules released by osteoblasts in the subchondral bone reach the articular cartilage where they promote cartilage degradation [35, 39].

Another change observed in our study was the reduction in the number of chondrocytes in all areas of the articular cartilage, indicating an increase in cell death. According to some studies, there are several types of cell death in articular cartilage: cell death through autophagy, cellular apoptosis, and various forms of necrosis [40]. The death of chondrocytes leads to hypocellularity, which is accompanied by a reduction in the capacity to produce the ECM and often does not form articular cartilage, but rather a fibrocartilaginous structure, which has inferior biomechanical and biochemical properties compared to articular cartilage [41–43].

Many studies showed that the mechanical stress environment of the joint is an important factor that influences chondrocyte activity *in vivo*. Understanding the role of specific mechanical phenomena in regulating chondrocyte metabolism and creating local inflammatory conditions can at least partially explain the etiopathogenesis of OA. There is increasing evidence indicating that cytokines and proinflammatory mediators associated with mechanical stress may be partially responsible for the catabolic events occurring in the OA cartilage [44].

In our study, using IHC techniques, we identified a moderate chronic inflammatory infiltrate in the synovial membrane and joint capsule, composed of lymphocytes and macrophages, secreting MMPs. Although it was traditionally considered non-inflammatory arthropathy, current studies suggest a significant role of the immune system components, particularly synovial inflammation, in the progression of OA [45, 46]. It is believed that the influx of immune cells into the synovium, the tissue that lines the joint capsule, mediates cartilage degradation through the production of inflammatory mediators [47]. These mediators promote the production of enzymes that degrade the cartilage matrix and reduce its synthetic activity. In addition, the synovium contributes to the pain associated with OA by promoting neurogenic inflammation mediated by neuropeptide-like substances [48, 49].

We showed that, sometimes, the repair of articular cartilage ruptures occurred through the presence of granulation tissue between the cartilaginous fragments, rich in fibroblasts and fibrillar collagen, aspects that denote the reduced regenerative capacity of the cartilage. Additionally, a large number of fibroblasts were found around the synovial membrane, which, in our opinion, participate in articular regenerative processes.

However, some studies showed that synovial fibroblasts are key players in the pathogenesis of OA. They promote the inflammatory environment of the disease by secreting proinflammatory cytokines, chemokines, and enzymes that degrade the ECM, collectively leading to cartilage erosion and joint destruction [50]. The use of PCNA showed a reduced capacity for chondrocyte multiplication compared to that of fibroblasts in the perisynovial joint capsule. Changes in the subchondral bone are correlated with changes in the articular cartilage, OA being characterized by cartilage degeneration, changes in the subchondral bone, osteophyte formation, and inflammation of the synovial tissue.

Normally, articular cartilage is a semi-rigid, elastic tissue that provides a smooth and lubricated surface for joint movement. It also plays a key role in the absorption and dissipation of mechanical forces to the underlying subchondral bone, so defects in articular cartilage are frequently associated with subchondral bone damage [35, 51]. Articular cartilage and subchondral bone form a closely composed functional unit called the "osteochondral junction" [52].

We observed increased bone OA in the femoral head in patients with hip OA. Thus, at the level of the subchondral plate, we identified areas of thickened bone in the early stages of the disease, as well as atrophic areas in the advanced stages. We must note that the majority of the patients were elderly individuals who also had a high degree of osteoporosis. The subchondral spongy bone was formed by thin trabeculae, with a barely visible endosteum, with areas of fissures and microfractures. The areolae were enlarged, sometimes with a cystic aspect, most often occupied by the adipose tissue.

Our observations are consistent with other studies that mentioned that increased bone remodeling and subchondral bone loss [53], thinning and increased porosity of the subchondral bone plate, a high incidence of trabecular microlesions, and a reduction in trabecular bone thickness were observed in the early stages of OA in humans [54–57].

Conclusions

At the level of the articular cartilage affected by OA, the absence of chondrocyte globules, a reduction in the number of chondrocytes, and very rare series of isogenic chondrocytes were observed. Chondrocytes appeared smaller, with reduced cytoplasm as a result of disrupted cellular metabolism. Frequently, empty chondrocytes were observed, resulting from the death of superficial chondrocytes, and the surface of the articular cartilage was often irregular and rough.

In advanced stages of the disease, cartilage fissures, joint surface deformation, cartilage matrix degradation, reduction in the number of chondrocytes, and changes in their morphology were identified.

In the case of severe forms of OA, areas of old cartilaginous fractures were sometimes identified, associated with the formation of granulation connective tissue with a reparative characteristic. At the level of the subchondral bone, a rarefaction of the bone trabeculae and a reduction in their thickness were observed, along with an increase in the size of the alveolar cavities and even the formation of cystic cavities. The myeloid tissue was replaced by unilocular adipose tissue. In some cases, an uneven hypertrophy of the subchondral bone plate was observed as a response to the reduction in thickness and alteration of the articular cartilage structure, while in other cases, a reduction in the thickness of the subchondral bone plate was noted.

At the level of the femoral head's bone tissue, we observed endosteal hypertrophy, absence of bone remodeling processes, cracks or even fractures of the bony trabeculae, and ectopic osteogenesis processes, either endochondral or desmal, which underlie the development of osteophytes.

The synovium presented itself as a heterogeneous connective tissue that contained immune cells, vascular endothelial cells, fibroblasts, adipocytes, and possibly other mesenchymal stromal cells.

The study of the inflammatory infiltrate, using IHC techniques, highlighted the presence of T-lymphocytes, B-lymphocytes, and macrophages, cells capable of synthesizing and releasing MMPs that intervene in the degradation of articular cartilage. The exploration of the cell multiplication capacity using PCNA showed that, at the level of articular cartilage, there are few cells (chondrocytes) capable of multiplication, while in the synovium, there are numerous young fibroblasts capable of mitotic divisions.

Conflict of interests

The authors declare that they have no conflict of interests.

Institutional Review Board Statement

The study was conducted according with the Declaration of Helsinki and was approved by the Ethics Committee of the University of Medicine and Pharmacy of Craiova, Romania (Approval No. 131/26.02.2025).

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