ORIGINAL ARTICLE

HISTOCHEMICAL MODIFICATIONS IN THE FEMORAL HEAD ARTICULAR CARTILAGE AFTER EXPERIMENTAL HIP DISLOCATION

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ABSTRACT

Background. The developmental hip dysplasia, if not treated in due time, may lead to regional modifications that even surgical intervention cannot solve. **Objective**. In this article we present an experimental study on three groups of 3-weeks-old rabbits with induced hip dislocation, our purpose being to evaluate how much can be the treatment delayed without compromising the hip, and at what point the histological modifications become irreversib**le. Materials and Methods:** in the three groups of 3-weeks-old rabbits the dislocation was maintained for different periods of time: 2 days, 7 days, and 2 weeks. The hip dislocation was then orthopedically reduced, and the reduction was maintained for another 2 days, 7 days, and 2 weeks respectively. The histological and histochemical changes in the articular cartilage of the femoral head were assessed on biopsies taken before the reduction and after the contention period, and in comparison to the control samples taken from the opposite hip. **Results.** At the end of the experiment there were no notable differences between the experimental and control specimens for the first group. In the second experimental group, some of the changes were maintained in two of the cases, while in the third group, where the most notable changes took place, the microscopic alterations showed only some remission by the end of the experiment. **Conclusion:** We show that the degree of morphological and histochemical changes varies with the time elapsed until orthopedic treatment application.
Keywords: Experimental hip dislocation, Articular cartilage of the femoral head, Alcian blue - Saphranin, Masson`s trichrome.

INTRODUCTION

The developmental dysplasia of the hip (DDH) is a term that comprises various clinical entities that include dysplasia, subluxation or luxation. The modified bone position in the hip joint is responsible for the articular cartilage damage that, if the disease is not addressed properly, may become irreversible. In some cases, especially in partially dislocated or hip subluxation, there is the risk of early onset of osteoarthrosis, even in the second decade of life (1). Early diagnosis of DDH, followed by early adequate treatment, is meant to prevent severe complications. With the aid of ultrasonography, the disease is detectable in the newborn children with incidences ranging from 0.1-3.4% (2). Still, there is always the risk of over diagnosing the DDH, and applying unnecessary treatment: up to 90% of the unstable hips diagnosed in the neonatal period spontaneously stabilize during the first month of life (3). But it remains the other 10% where the changes that occur in the modified hip joint are followed by changes in the articular cartilage. So it becomes important to know how much we can delay the treatment without compromising the hip, and at what point the histological modifications become irreversible.

From the histological point of view, the normal articular cartilage is characterized by an abundant homogenous extracellular matrix with relatively sparse cells located in well-defined spaces called lacunae. It is structured in four layers (or zones): superficial, intermediate, deep, and calcified, the junction between the deep zone and the calcified cartilage being marked by a line called the tidemark. In the superficial layer, the cells are flat. In the intermediate zone, the cells have a tendency to form radial groups that apparently follow the pattern of collagen disposition. In the deep zone, the cells are hypertrophied; and in the calcified zone (i.e., the zone adjacent to the bone), the cells are nonviable and the matrix is heavily calcified (4).

The special mechanical properties of the articular cartilage are determined by the extracellular matrix components secreted by the chondrocytes - type II collagen, proteoglycans (PG) and glycosaminoglycans (GAGs)-, and their distribution: the superficial zone has the highest collagen proportion, and the intermediate zone – the highest PG proportion (5). Most histological and histochemical studies regarding the DDH, focus on the modifications that appear in the acetabular cartilage, with only little information on the articular cartilage of the femoral head. In this article we present the histological and histochemical results of our experimental studies on femoral heads in rabbits with induced hip dislocation.

MATERIAL AND METHODS

For this study we chose rabbits because there is an anatomical similitude between the rabbit and human hip joints and, also, because the orthopedic and surgical maneuvers are easy to perform. All orthopedic and surgical maneuvers (biopsies) were done under general anesthesia with isoflurane, respecting the general guidelines of the Ethical Comity of our University. We used 15 rabbits from the "Pius Branzeu" breeding Centre. All 3 weeks-old rabbits were subject to orthopedic unilateral (left) coxo-femoral luxation. The lot was then divided into three groups of 5 rabbits each: in the first group the luxation was orthopedically reduced after 2 days, in the second - after 7 days, and in the third - after 14 days. Two rabbits from the last group needed surgical reduction. The splint contention was preserved for different periods of time in the three groups: 2 days for the first group, 7 for the second, and 14 days respectively, for the third group. Before the reduction and after the contention period, for each rabbit were taken biopsies from the articular cartilage of the dislocated femoral head, under radiological control. Also, before reduction, other biopsies were taken from the opposite, healthy femoral head for the control specimens. During the experiment two of the rabbits died from causes not connected to the current experiment: one from the third group – 28 days in the experiment (incident during anesthesia), and one from the second group – 7 days in the experiment. In these cases the entire femoral head was harvested. After 24/48 hours fixation in 10% buffered formalin, the biopsies /femoral heads, were embedded in paraffin. From each specimen 3 sections, 5 micrometer thick were made. After standard dewaxing and rehydration one slide was stained with haematoxylin and eosin (HE) for morphological diagnosis. The second slide was stained with Masson`s trichrome for mature and immature collagen distribution, and the third one with Alcian Blue-Safranin at pH 1.42 for PGs and GAGs.

The slides were examined with a Nikon Eclipse i80 microscope with10X, 20X, and 40X objectives. For each specimen three images were taken (one for each staining method), with a DS-U2 digital camera attached to a Nikon Eclipse i80 microscope.

RESULTS

All specimens from the experiment were compared to the controls and the results were enunciated accordingly. For Masson`s trichrome we obtained purple nuclei, pale pink cytoplasms, and blue interterritorial matrix. Some red fibrillar deposits were observed in the experimental group. For the Alcian Blue-Safranin staining we obtained two kinds of results: some of the samples showed both orange-red, safraninophilic areas, and blue, alcianophilic areas, and others, only alcianophilic areas.

For the first group, two days after the hip dislocation, HE staining showed minimal changes in the central zone of the femoral head articular cartilage: a slightly more intensely stained territorial matrix, and larger isogenous groups (4-5 cells) than in the control specimen. In the same zone, Masson`s trichrome showed a more intense reaction for collagen fibers, and the extracellular matrix had a pale positive reaction for safranin. In one case there was no positive reaction for safranin, and in another – there was an intense positive reaction in the pericellular matrix. The second set of biopsies, taken two days after orthopedic reduction, showed no notable morphological or histochemical differences between the control and the experimental specimens.

In the second group of rabbits, in three of the cases there were small irregularities of the cartilage surface (as shown in Figure 1a). Also, in the same cases, there were matrix areas, located between the intermediate and deep zone of the articular cartilage,that were free of chondrocytes,. Only two of the specimens had intense safraninophilic deep zone matrix. After the seven days contention, in two of the cases the cartilage surface still presented small irregularities, with empty lacunae opening directly onto the surface. In three of the cases the chondrocytes in the intermediate zone had intensely safranin positive pericellular matrix, and alcian positive territorial matrix (as shown in Figure 1b).

On the specimens from the third group we observed the presence of a continuous deposit of red, immature collagen fibers at the surface of the articular cartilage. In some places, under the immature collagen coat, the chondroblasts were larger, and the cartilaginous extracellular matrix lost its hyaline aspect and became fibrillar (Figure 2). These fibrillar deposits were oriented oblique/perpendicular to the surface. In the deep zone of the articular cartilage the tidemark was interrupted and in some places absent. Vascular canals, emerging from the subchondral bone zone, were penetrating the cartilage up to the intermediate zone. These canals were lined by osteoblasts, and were surrounded by calcified cartilaginous matrix in which some viable chondrocytes were caught (Figure 3a and Figure 3b). The extracellular matrix was negative for safranin. The alcianophilia around the canals was in some zones absent and in others pale (Figure 3c). Two weeks after orthopedic/ surgical reduction and containment, the articular cartilage surface had small irregularities, but no immature collagen fibers were observed. The canals in the intermediate zone were filled with a fibrillar material (thick bundles of immature collagen) and some cells. In the deep zone the cells were arranged in large isogenous groups (Figure 4a) and a tidemark was also present. The interterritorial matrix in this area stained pale for safranin, and the territorial matrix was alcianophilic. With the same staining, the walls of the vascular canals were partially intensely alcianophilic, precisely contoured, and partially – pale, vaguely contoured (Figure 4b).

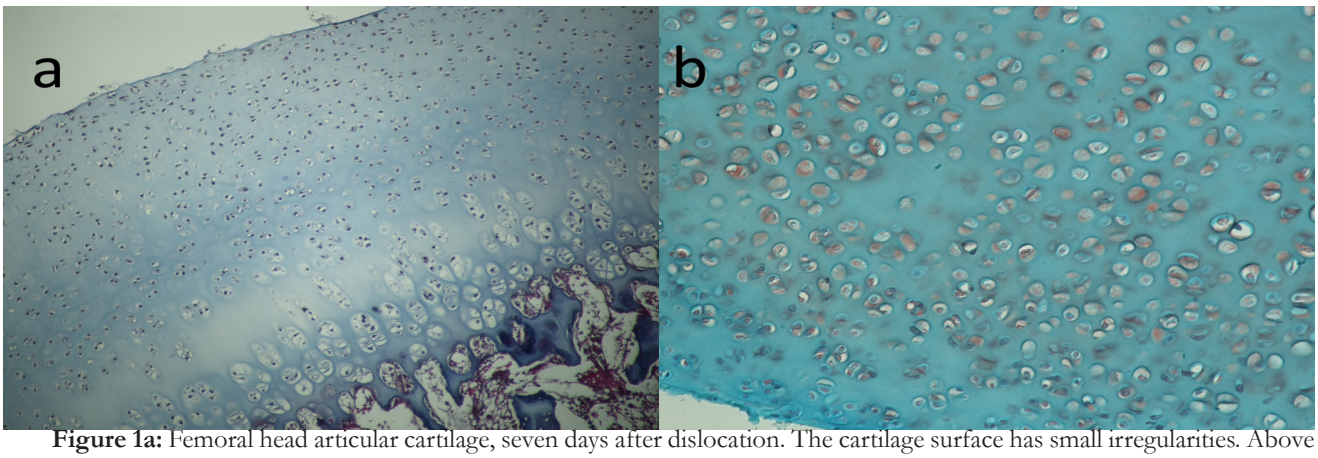

Figure 1a: Femoral head articular cartilage, seven days after dislocation. The cartilage surface has small irregularities. Above the deep zone there ae matrix areas free of cells. Masson`s tricrome X10.**Figure 1b:** Femoral head articular cartilage, seven days after repositioning. The chondrocytes in the intermediate zone show signs of intense PG and GAG synthesis –safranin positive pericellular matrix, and alcianophilic territorial matrix. Alcian-Blue – Safranin X20

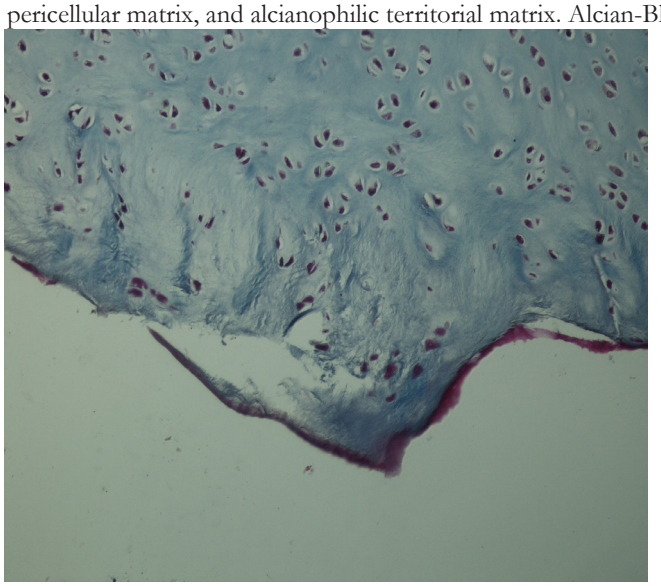

Figure 2:
Femoral head articular cartilage, fourteen days after dislocation. A collagen deposit is seen on the articular surface. The external cartilage matrix is no longer hyaline, but is fibrilar in aspect. Masson`s trichrome X40

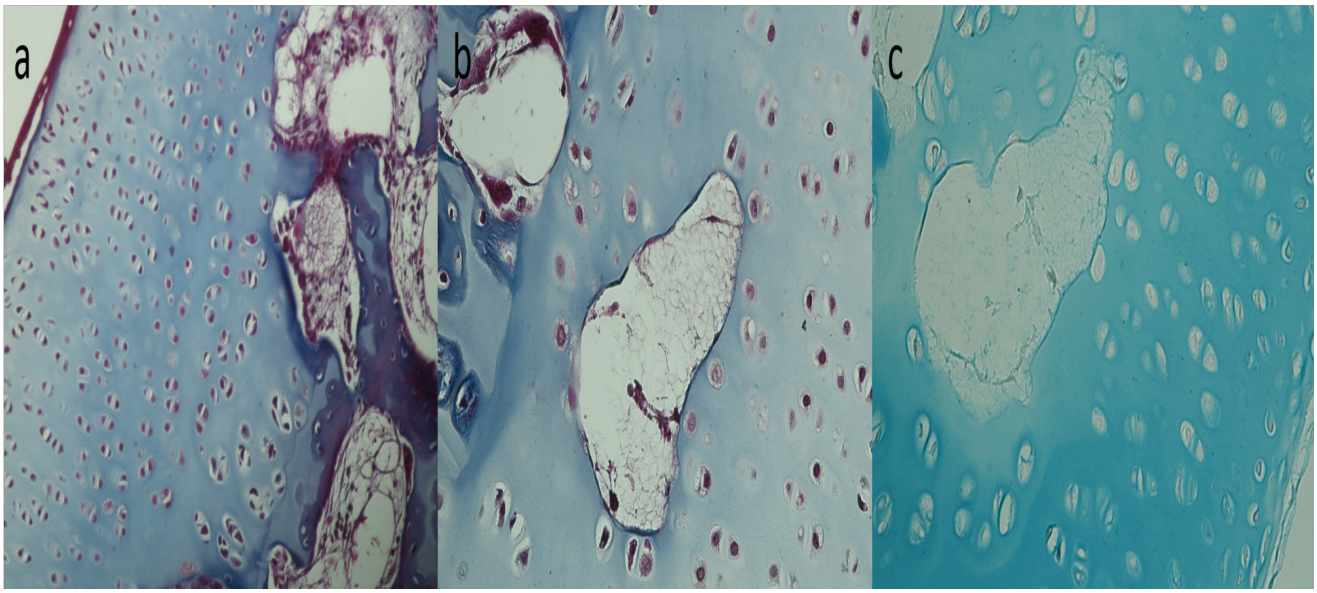

Figure 3a: Femoral head articular cartilage, fourteen days after dislocation. Vascular canals lined by osteoblasts at the advancing front penetrate the cartilage from inside. These canals are surrounded by calcified matrix that includes groups of chondrocytes. Masson`s trichrome X20.**Figure 3b:** Femoral head articular cartilage, fourteen days after dislocation. Vascular canal located in the intermediate zone that engulfs the surrounding chondrocytes. The surrounding matrix contains mostly chondrocytes with large, euchromatic, nucleolated nuclei, and also dividing chondrocytes. Masson`s trichrome X40.**Figure 3c:** Femoral head articular cartilage, fourteen days after dislocation. Notice the surrounding matrix of a canal that shows zones with a very weak reaction for Alcian-Blue (where the cartilage matrix and lacune are eroded). The interterritorial surrounding matrix stains paler than the rest; the territorial matrix in this zone is intensely stained. No safranin –positive reaction was observed. Alcian – Blue Safranin, X40.

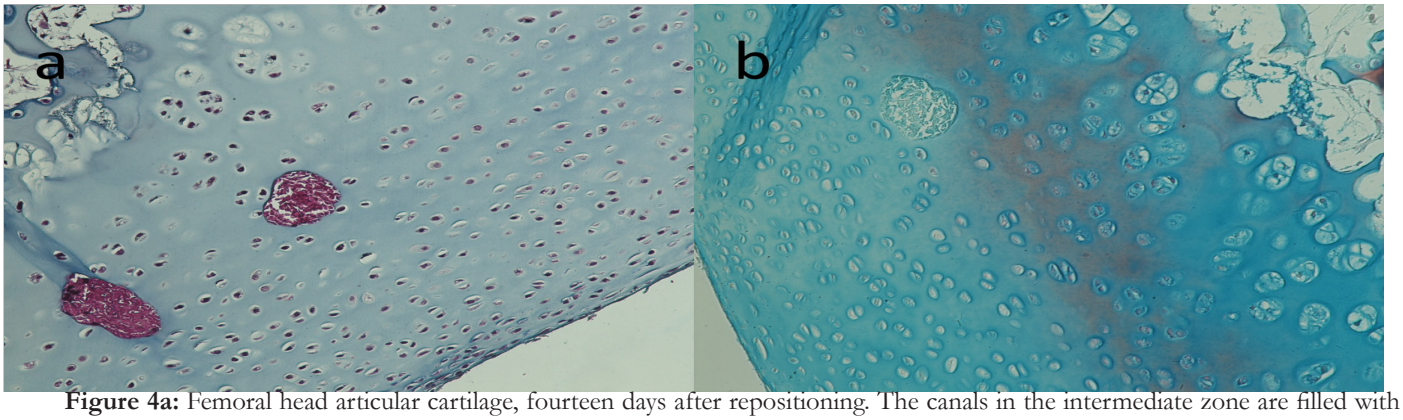

Figure 4a: Femoral head articular cartilage, fourteen days after repositioning. The canals in the intermediate zone are filled with dark red fibrilar deposits. On their contour, inconstantly, the surrounding matrix presents dark blue deposits. Masson`s trichrome X20.
Figure 4b: Femoral head articular cartilage, fourteen days after repositioning. The interterritorial matrix in the lower intermediate and in the deep zone is positive for safranin. The matrix surrounding the canal is unevenly alcianophilic. Alcian – Blue Safrani , X20

DISCUSSIONS

In the hip joint, both bony acetabulum and femoral head are covered by hyaline cartilage that ensures a smooth contact, and frictionless motion. Due to its structure and composition, the articular hyaline cartilage may deform under mechanical stress, and recovers its shape after the stress is removed (6). If the stress is prolonged, in the articular cartilage appear changes that are the result of either altered molecular synthesis in the chondrocytes or of enzymatic breakdown of the matrix components, and are more apparent in the surface cartilage (7). In any case, the result is a modification in either, or both major components of the extracellular matrix: collagen type II and PG both secreted by the chondrocytes. Being avascular, the cartilage is nourished by diffusion from the synovial fluid and has a very low healing capacity. Only those defects that result in low amount loss of extracellular components can be compensated by the chondrocytes. More extensive or prolonged damage exceed the reparative capacity of the chondrocytes and results in scar formation, namely turning of the hyaline into fibrous cartilage or, if the damage reaches the cellular level, the necrosis of the cartilage (4).

In our study we tried to assess histochemically the main components of the articular cartilage matrix, and their changes according to the time lapse between the occurence of hip dislocation and repositioning of the femoral head. If in the first group after two days the matrix changes were minimal, in the second group (after seven days) they were more notable, and in the third group (after fourteen days) they were dramatic.

For collagen assessment we used Masson`s trichrome (8). With the exception of the third group samples, we could not obtain valuable information about collagen distribution.

Normally the articular cartilage has no perichondrium, and the collagen fibers in its matrix are oriented vertical, with the exception of the surface layer where they are horizontal (9). In the third group of rabbits, in which the femoral head dislocation was maintained two weeks, the articular cartilage developed a fibrillar coat at the surface -what we considered to be a pseudoperichondrium. This coat made up of immature collagen bundles was accompanied by visible mature thick collagen fiber deposits in the peripheral matrix. Because they disappeared two weeks after repositioning and splint containment, these modifications were not considered irreversible. Still, the intense collagen synthesis suggests that a prolonged delay of treatment would have resulted in lesions similar to those seen in osteoarthritis (10).

The use of combined Alcian Blue-Safranin staining for differentiating the lightly sulfated alcianophilic GAGs from the strongly sulfated safraninophilic GAGs in cartilage matrix is controversial (11). If the intensity of safranin reaction is proportional with the PG content in the cartilage matrix, the reaction for alcian-blue was found to depend on a series of factors as: pH, concentration of staining solution, duration of staining, temperature (12). Musumeci at al (13) stated that safranin O is commonly used for GAGs, and alcian-blue –for PG. Our results are consistent with the literature: in reparing articular cartilage of the second group in our experiment, the chondrocytes in the intermediate zone were surrounded by an intense safraninophilic pericellular matrix. We interpreted this finding as a sign that the chondrocytes have an intense sulfated GAG synthesis in order to compensate the unbalance created by femoral head dislocation. When stained with Alcian –Blue Safranin, all of our control samples showed an intense positive reaction in the interterritorial matrix of intermediate zone in the articular cartilage. This reaction was obtained also on the experimental samples at the end of the experiment, with the exception of the third group, where the positive reaction was partially present also in the deep zone of the reparing cartilage. Despite the fact that all samples were processed in the same conditions, not all of them had a positive reaction for safranin. In the case of the third group we interpreted this fact as a result of prolonged unload of the femoral head, as it is known that immobilization or unloading of a joint results in decreased GAG synthesis (4).

We found that the cartilage matrix modifications varied according to the period of time until the orthopedic/surgical reduction, being more dramatic in the third group where the treatment was established after two weeks. In fact in this group, two weeks after repositioning, the cartilage surface was not yet completely repaired. Also the vascular canals, even if filled with fibrous material, were still in place, as a future source for scar healing and calcification.

In conclusion, after two weeks of experimentally induced hip dislocation we found borderline matrix modifications. If the dislocation would have been maintained for a longer period of time, even a few days (taking in consideration the major differences between the second and the third studied groups), the changes would have been permanent. The fact that not all the rabbits in a group showed the same degree of matrix modifications, even if the experiment conditions were identical for each group, it means that also individual factors are involved.

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CONTRIBUTIONS

Experiment planning (AIP, ESB), execution (AIP, ANP, MDT, CI, RD, LS), study analysis (CI, ESB), histology (SS), manuscript design (AIP, SS, LM), manuscript writing (SS), proofreading (AIP, LM, ESB).

DECLARATION OF CONFLICTING INTERESTS:

The authors declare they have no conflicting interests with regard to this research or publication of this article.

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