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CHANGES IN THE GUINEA PIG'S SHOULDER AFTER CREATION OF SOFT TISSUES CONTRACTURE. EXPERIMENTAL STUDY

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SUMMARY

We studied the role of abnormal biomechanics in the development of osteoarthritis in the shoulder joint. An experiment was done on 30 guinea pigs, five months of age, weighing 380 to 420 grams. We created a contracture to produce a model of surgical limitation to joint mobility. On 30-th, 60-th and 90-th days from the start of the experiment, we used histologic methods and electronic microscopy to investigate the morphological and structural changes of articular soft tissues, cartilage surfaces and bone epiphysis. We observed a rapid progression of cartilage degenerative changes and subchondral ossification from days 60 to 90. Our data proved the hypothesis that joint contracture can be an independent factor in the appearance and progression of osteoarthritis of the shoulder joint.

Keywords: experiment, guinea pig, contracture, osteoarthritis, shoulder joint

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ZMIANY W STAWIE RAMIENNYM ŚWINKI MORSKIEJ PO EKSPERYMENTALNYM WYTWORZENIU PRZYKURCZU TKANEK MIĘKKICH

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STRESZCZENIE

W pracy badano rolę nieprawidłowych parametrów biomechanicznych stawu ramiennego w rozwoju choroby zwyrodnieniowej. Eksperyment przeprowadzono na 5-miesięcznych świnkach morskich o wadze od 380 do 420 gramów. Stworzono przykurcz, aby zasymulować model chirurgicznego ograniczenia ruchomości stawów. W 30, 60 i 90 dniu od rozpoczęcia eksperymentu, wykorzystano metody histologiczne i mikroskopię elektronową do zbadania zmian morfologicznych i strukturalnych miękkich tkanek stawowych, powierzchni chrząstki i nasady kości. Obserwowano szybki postęp zmian zwyrodnieniowych chrząstki i kostnienia podchrzęstnego w dniach od 60 do 90. Nasze dane potwierdziły hipotezę, że przykurcz może być niezależnym czynnikiem w pojawieniu się i postępie choroby zwyrodnieniowej stawu ramiennego.

Słowa kluczowe: eksperyment, świnka morska, przykurcz, choroba zwyrodnieniowa stawów, staw ramienny

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Introduction

Osteoarthritis is the most common abnormality of the shoulder joint, leading to serious consequences (Birrell *et al.* 2011, Chilleni and Franceschini 2013). Many of current treatments for shoulder joint disorders are often ineffective and are accompanied by a large number of complications. This may be explained by delayed treatment and insufficient assessment of pathogenic components in the onset and progression of abnormal changes. Today, traumatic injury to capsular ligaments of the joint and affected joint mobility are considered one of principal factors in the development of arthrosis (Reuther *et al.* 2014, Prieto-Alhambra *et al.* 2014). However, the role of abnormal biomechanics in the development of osteoarthritis of the shoulder joint as the pathogenic monofactor remains unidentified.

Aim

The aim was to establish the specifics of the onset and progression of structural changes of the shoulder joint under the conditions of simulated abnormal biomechanics and contractures of the shoulder joint as the monofactor in formation of deforming osteoarthritis.

Material and methods

Experimental studies were done on 30 guinea pigs (5 months old, weight: 380 to 420 g). We made a shoulder contracture by suturing the rotator cuff interval with a bioabsorbable stitch. The joint was not opened up. Before the surgical procedures, the animals were anesthetized with thiopental sodium (50–60 mg/kg, i.p.). We used surgical technique described in article of Kramer E.J. (Kramer *et al.* 2013).

The animals were taken out of the experiment on day 30, 60 and 90 after the procedure by injecting a lethal dose of thiopental sodium.

Histologic study

Shoulder joints were fixed in 10% formalin solution in 0.1 M phosphate buffer (PB; pH 7.4) for 24 hours at 4°C, and then washed with running water. Humerus and scapula were decalcinated in 5% EDTA at pH 6.0–6.5. Decalcification in the first 24 hours was done at 4°C, then the solution was replaced and further decalcification was done at room temperature for 20–30 days. The solution was changed every 5 days. We obtained 12–15-micron cryosections from the decalcinated shoulder joint and further stained them with picrofucsin, toluidine blue and hematoxylin-eosin.

Morphometric study

Morphometric analysis refers to the quantitative analysis of degenerative changes of the articular surface of the humeral head and glenoid. Thickness of the articular surface was measured in all histologic sections along its margin in 20 different places. Photomicrographs were obtained, using Olympus BX 51 microscope and CarlZeiss software (AxioVision SE64 Rel.4.9.1) zoomed by $\times 200$ and $\times 400$.

Scanning electron microscopy

For the qualitative and quantitative analysis of degenerative changes of the articular surfaces, some samples were analysed in a scanning electron microscope (SEM). Samples were dehydrated in ethanol solutions of increasing concentrations (25% – 50% – 75% – 100%). Then materials were dried at the critical point of CO₂ in Samdri-780A. Dried samples were covered with gold (thickness of 15 nm) in a vacuum coating machine (Gatan 682 PECS). SEM studies were performed on a microscope TESCAN Mira 3 LMU at the electron microscopy laboratory (NanoMedTech, Kiev, Ukraine; head of the laboratory – Skoryk M.A.).

Statistical analysis

Statistical analysis was performed, using the Student t-test. Distinctions were considered

reliable, if $p < 0.05$. Data were presented as $M \pm SD$.

Bioethics

All experimental manipulations were done according to the provisions of the European convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986), Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes, Ukrainian Law No 3447-IV On the protection of animals against cruelty, and Guide for the care and use of laboratory animals.

Results

Deforming osteoarthritis is characterized in histologic studies by a loss of cartilage surface against degenerative changes of the hyaline cartilage on the joint surface, and ossification of the subepiphyseal region. The dynamics of the onset of deformation changes and their progression has not yet been established. To this effect, we have analysed characteristics of osteoarthritis formation in different time periods.

In the control (intact) animals, the epiphyseal surface of the humeral head and glenoid was represented by a layer of the hyaline cartilage, the latter was surrounded by the perichondrium on its external margin. The perichondrium is involved in the formation of the joint capsule. In the intact hyaline cartilage, basic substance of mucoid and fibrous elements (GAGs and collagen) dominated. This cartilage comprises isogenic groups of chondrocytes, the density and volume of which increase from the contacting surface to the subepiphyseal bone. Tendons were attached to the joint capsule. In the control group of animals, morphological signs of degenerative changes on the articular surface, subepiphyseal region, tendons or capsular were not found. SEM showed smooth articular surface without any deformations or traumatic injuries.

On day 30 after the surgery, we observed a contracture of the shoulder joint, limiting its biomechanics, in the experimental group of animals (on 30 guinea pigs, five months of age, weighing 380 to 420 grams). Reduced mobility of the shoulder joint was recorded until the experiment ended. On days 30, 60 and 90 the animals were taken out from the experiment. Results of histological and ultrastructural study of the humeral head of and glenoid depression were analyzed. SEM study was performed done to analyze topography of deformation osteoarthritis SEM study was done to analyze topography of deformation osteoarthritis. To identify changes in chemical chondromucoid activity, or glycosaminoglycans content, slices were stained with toluidine blue. Morphological changes of articular surfaces of the humeroscapular joint were evaluated by applying morphometric technique and using cryosections that were stained with hematoxylin and picrofucine. It expressed signs of structural changes in the hyaline cartilage and subepiphyseal bone in 30 days after the surgery, which are typical for osteoarthritis. We recorded deformation of the cartilage surface, focal reducing of cartilage thickness in the lower and front pole of the humeral head and the central area of glenoid, and to a lesser degree in the anterosuperior, front and upper pole (Fig. 1). From the side of epiphyseal bone, we also recorded sprouting of blood vessels into the deep zone of cartilage and paravasal ossification, and reduction of acid GAGs.

On day 60 after the surgery, structural changes progressed on both articular surfaces. The density of ossification areas increased on the anteroinferior surface and to a lesser degree on the posteriorinferior surface of the humeral head and the entire central zone of the glenoid.

In 90 days after the surgery, the access zone on the macro level was characterized by a severe reorganization of the connective tissue and vascular elements, and increase

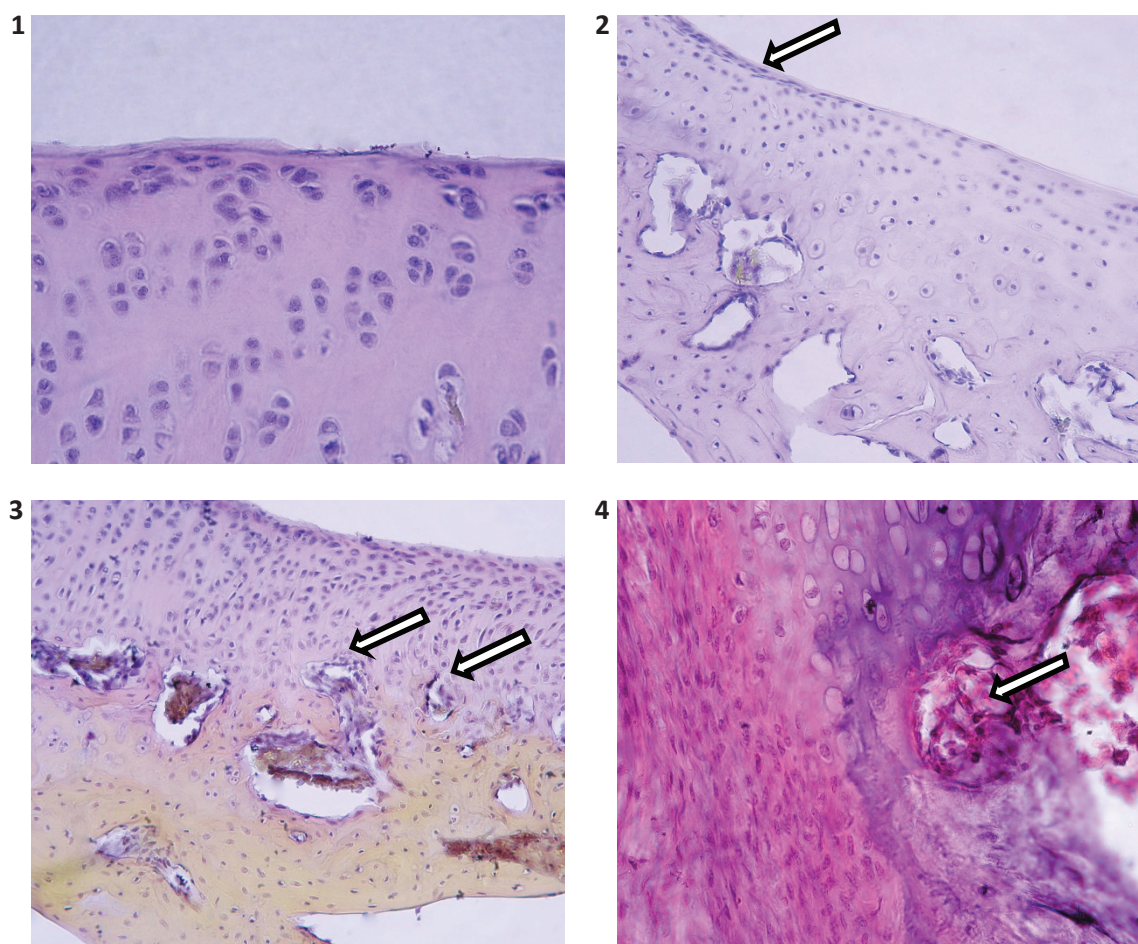


Figure 1. Histological changes of cartilage surface of the shoulder joint against simulated deformation osteoarthritis. We determined degenerative changes of articular surfaces with decreasing thickness cartilage framework, degenerative changes of isogenic groups of chondrocytes, blood vessels reaction and subchondral ossification. Note: 1 – intact articular surface of the humeral head in the control group of animals (hematoxylin and picrofucine stain, $\times 400$); 2 – focal reduction of thickness (\Leftarrow) of the articular surface in 30 days after the surgery (hematoxylin and picrofucine stain, $\times 200$); 3 – subchondral ossification (\Leftarrow) on the central side of the joint surface in 60 days after the surgery (hematoxylin and picrofucine stain, $\times 200$); 4 – paravascular subchondral ossification (\Leftarrow) on the anteroinferior surface of the humeral head in 90 days after surgery (hematoxylin and eosin stain, $\times 400$).

of connective tissue density (newly fibrous tissue, fat tissue). As to the capsular ligament apparatus of joint, we noticed easing of the paraarticular tissue, hyperemia and expansion of small veins and arteries, formation of the fibrous connective tissue around blood vessels, joint capsule, and destructive changes of periosteum and capsules. On the anteroinferior and posterior inferior surface, an increase in joint capsule thickness, density of activated fibroblasts, connective tissue invasion (inferior fibrovascular tissue) in stromal elements of local muscles were found.

In the study of the topography and the degree of degenerative changes, much attention was paid to the study of the joint surface area, as because of its location it feels the bulk of the load, in case of abnormal biomechanics, and, therefore, quickly loses elasticity, density and undergoes degeneration.

Histology of anteroinferior and posterior joint surfaces is characterized by signs of focal or diffuse destruction of the cartilage. A reduction of cartilage thickness in a large area of the joint surface of the head of the humerus and scapula depression was

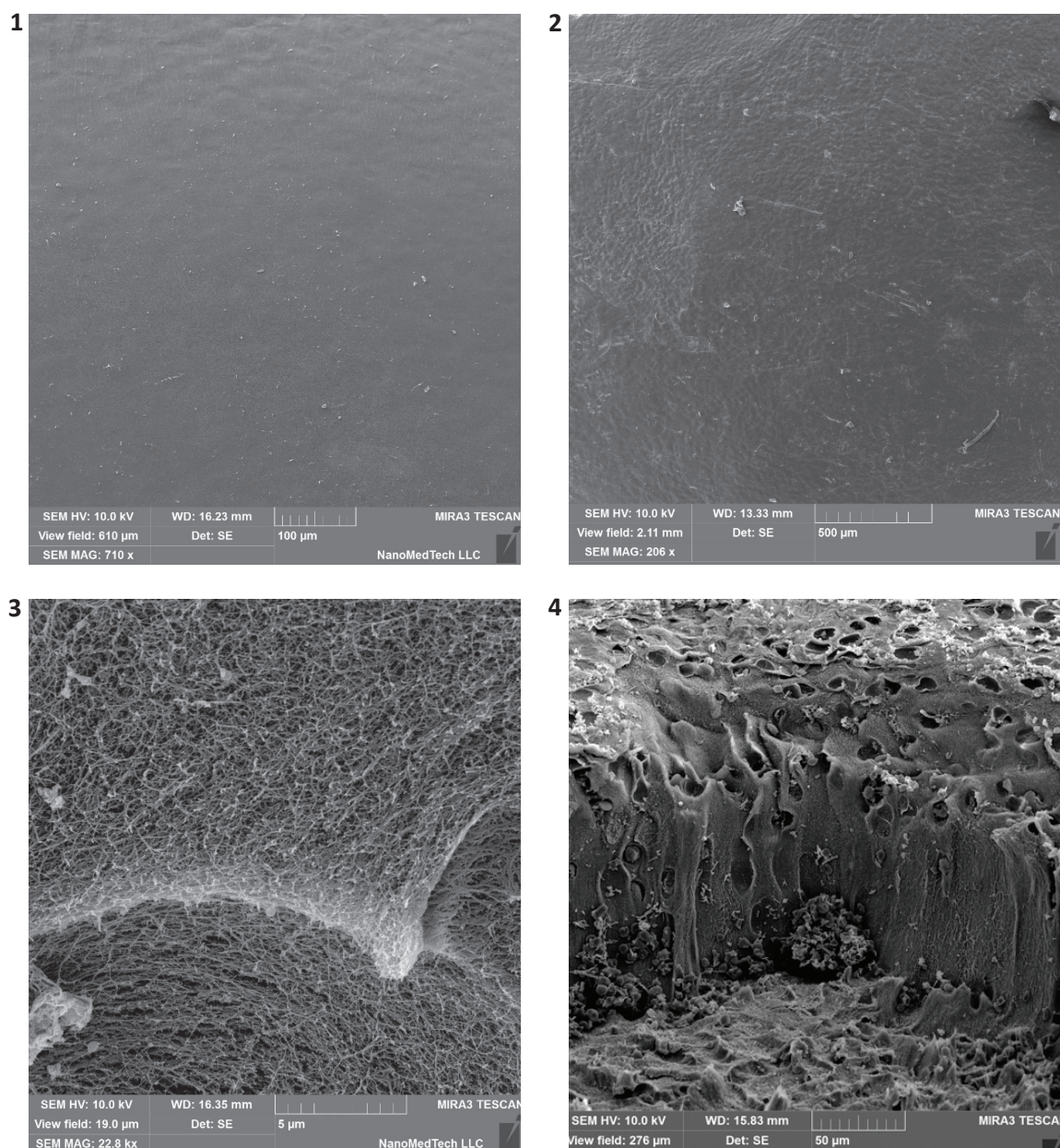


Figure 2. The results of SEM study of the head articular surface of the humeral head against simulated deformation osteoarthritis. We registered degenerative changes of hondromucoid and chondrocytes. Note: 1 – structurally intact articular surface; 2 – minor affected articular surface in 30 days after the surgery; 3 – hondromucoid disruption in 60 days after the surgery; 4 – degenerative changes of the articular surface in 90 days after the surgery, degenerative changes and loss of the hyaline cartilage.

noticed. A deep zone of the cartilage surface contained newly perivascular ossification foci. General morphological organization of the joint surface on day 90 of our observation remained intact; however, it revealed a sharp regression of the damaged cartilage (Figs. 1, 2). The predominant number of isogenic chondroblast groups expressed edema and necrosis. Ossification

zones spread from the epiphyseal center of the subchondral bone to its surface. Blood vessels with osteogenic cells were formed in the created gaps. The surrounding hondromukoyid was affected by compaction and ossification. At the same time, a significant change of the epiphyseal bone outline was observed. Degeneration of the basic cartilage substance was characterized by

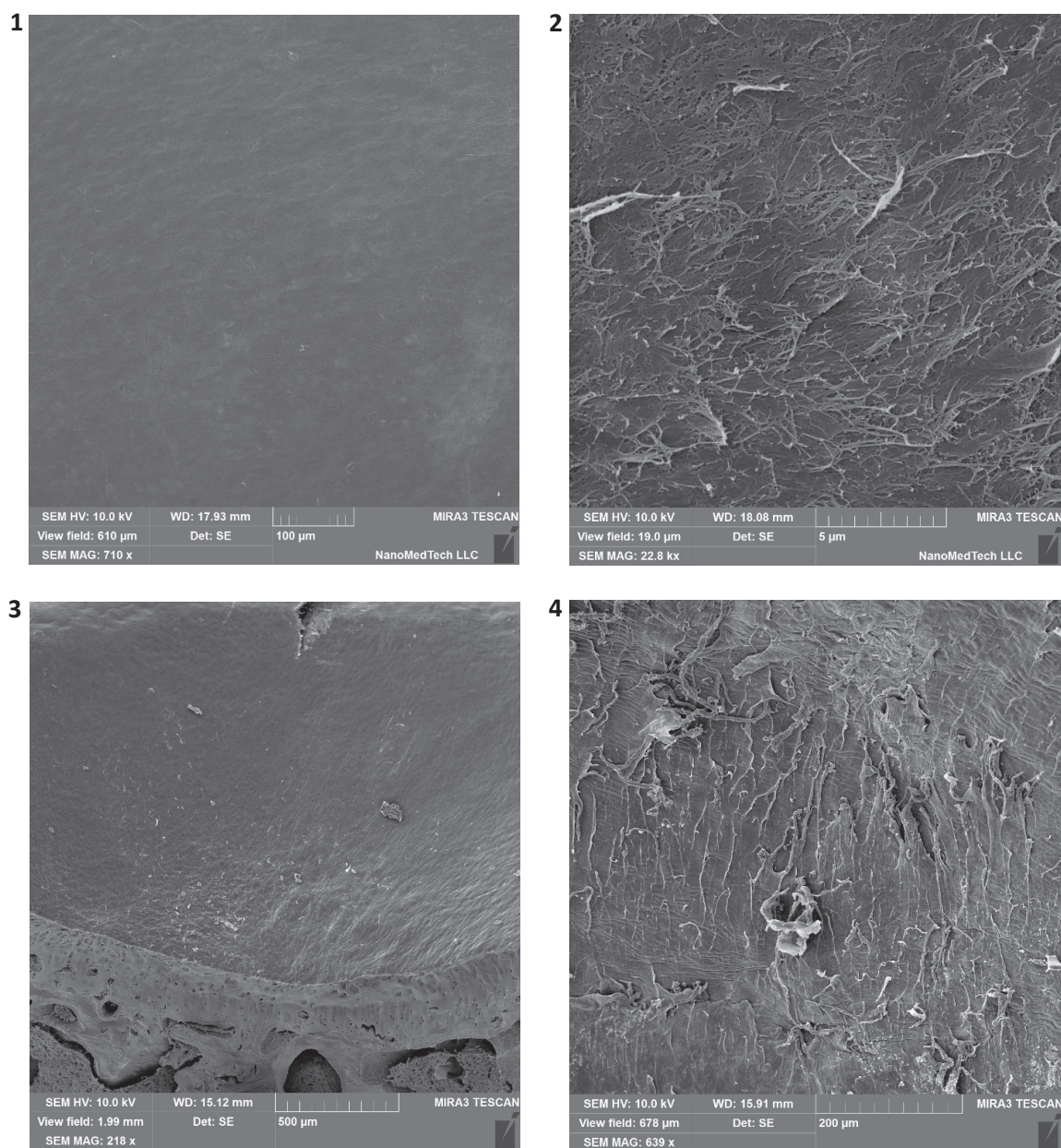


Figure 3. The results of SEM study of the articular surface of the glenoid against simulated deformation osteoarthritis. We determined deformation and abrasion of articular surfaces in the upper, anterosuperior and central pole of the glenoid. Note: 1 – structurally intact articular surface; 2 – abrasion of the surface layer in 30 days after the surgery; 3 – deformation zones in the central pole in 60 days after the surgery; 4 – progressive deformation and degenerative changes of the glenoid surface in 90 days after the surgery.

a sharp change in the joint surface pattern. The latter was clearly confirmed by SEM (Figs. 2, 3).

Discussion

Thus, at the ultrastructural level a loss of cartilage surface area in the background of its deformation, reducing the amount of the basic hondromukoyid substance and

density of fibrous cartilage components were found. Starting from day 60 of our observation, a sharp increase in the degree of degenerative changes in the shoulder joint that progressed from the central zone of the joints surface to the external circuit was recorded. Described histopathological changes were confirmed by morphometric analysis (Fig. 4), and the dominant

frequency of structural defects is given in Table 1.

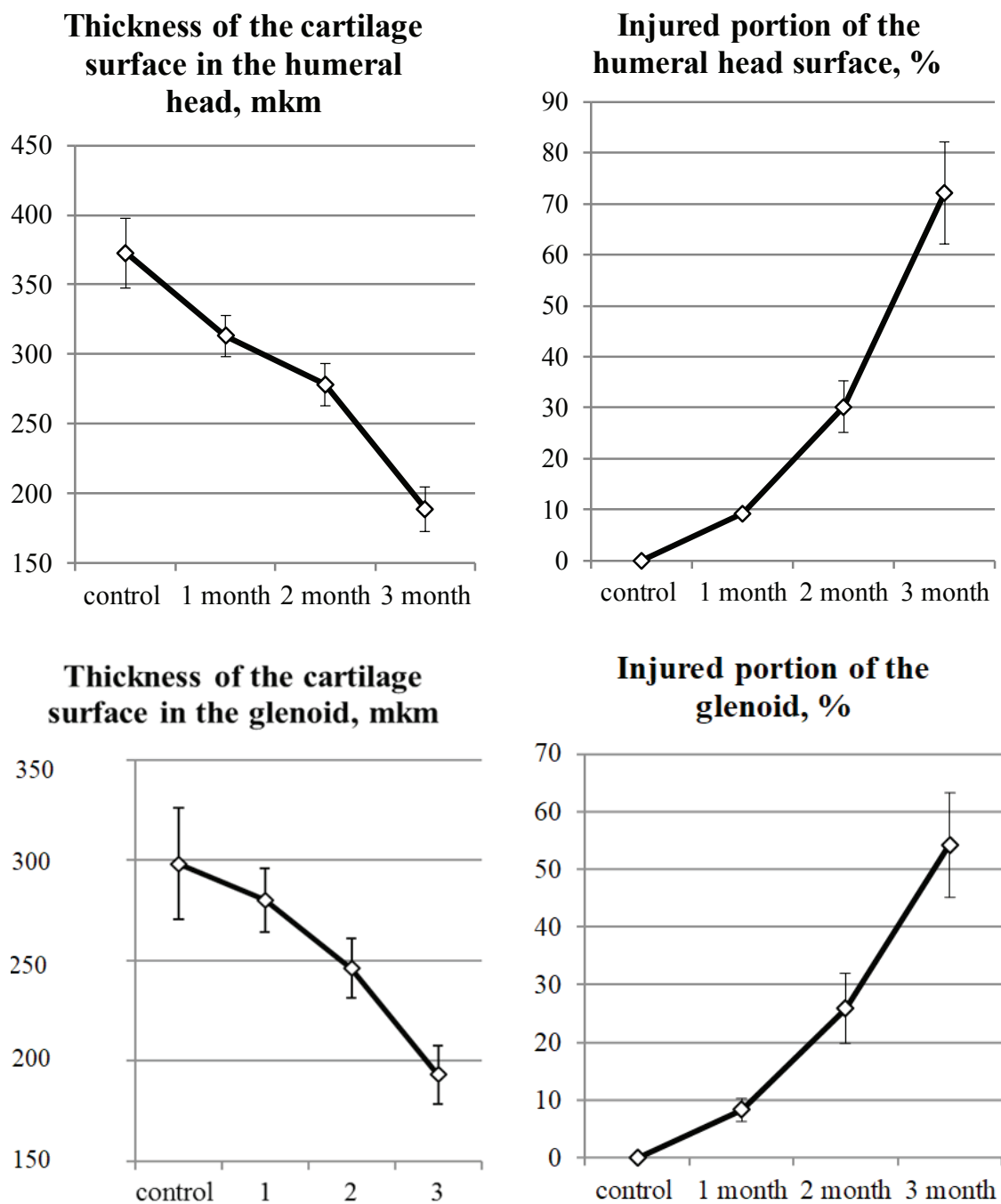


Figure 4. Dynamic morphometric characteristics of degenerative changes on the articular surface of the shoulder joint.

Table 1. Morphological changes of cartilage surface in shoulder joint.

Group	Histological signs of structural changes			
	Proliferative and degenerative changes in the joint capsule	Angiogenesis in the joint capsule	Dystrophic changes of the cartilage surface	Focal subepiphyseal ossification
Humeral head				
Control	—	—	—	—
1 month	2/5 40.0%	3/5 60.0%	2/5 40.0%	1/5 20.0%
2 months	3/5 60.0%	3/5 60.0%	6/5 60.0%	3/5 60.0%
3 months	9/9 100%	7/9 77.7%	5/9 55.5%	2/9 22.2%
Glenoid				
Control	—	—	—	—
1 month	2/5 40.0%	3/5 60.0%	2/5 40.0%	1/5 20.0%
2 months	3/5 60.0%	3/5 60.0%	5/5 100.0%	4/5 80.0%
3 months	9/9 100.0%	7/9 77.7%	7/9 77.7%	7/9 77.7%

Conclusions

Thus, the results of the experimental study confirmed the hypothesis that abnormal restriction of mobility of the shoulder joint and its abnormal biomechanics are independent factors in the onset of joint's osteoarthritis. Thus, as the results of our follow-up, the most critical period is 30–60 days, as long as the degenerative effects reached the most vulnerable ossification of the cartilage deep zone in this period. Cartilage exposures in the projection of the joint's friction zone surface and concomitant subepiphyseal ossification have caused the rapid progression of degenerative changes in the shoulder joint. Based on these data, we may consider the first 30 days as a therapeutic window for correcting the abnormal process.

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