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Histological features of articular cartilage and bone marrow reparative potential under conditions of coxarthrosis in patients with radiographic signs of epiphyseal dysplasia

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Coxarthrosis in patients with radiographic signs of epiphyseal dysplasia causes disturbances of social adaptation of this patients group at a young age and ensure the relevance of studying the problem of optimizing the orthopaedic treatment of this patients category. Objective. To define the tactics of orthopaedic treatment in such patient category based on study of morphological features of articular cartilage and osteogenic activity of bone marrow stem stromal cells. Materials and Methods. We have clinically examined 68 adult patients having coxarthrosis in the presence of radiological signs of epiphyseal dysplasia. In 52 cases we performed total hip and knee arthroplasty that allowed to obtain articular cartilage fragments for histological study and epiphysis bone fragments for study of reparative potential of the bone tissue. Results. In patients having coxarthrosis that evolves on the ground of epiphyseal dysplasia by histological and cultural studies we have obtained the data as for deep microstructural disorders in joint cartilage matrix organization as a result of modification of collagen mesh in patients having epiphyseal dysplasia. We have identified the fact of significantly increased bone marrow stem cells proliferative potential at significantly decreased quantity of colony forming fibroblast units in spongy volume unit in epiphysis zone in this patients group which indicates a threat of decompensation of reparatory bone potential risk. Conclusions. Pathological factors of increasingly progressing course of osteoarthritis in the presence of radiological signs of epiphyseal dysplasia are deep microstructural disorders of joint cartilage matrix organization as a result of modification of collagen mesh and consequent changes of epiphysis of the lower limbs form. There is no possibility of prevention and etiological therapy of coxarthrosis evolving from epiphyseal dysplasia, meanwhile there is a threat of decompensation of reparatory bone tissue potential in epiphysis zone in this patient category. Therefore, in patients with coxarthrosis and radiographic signs of epiphyseal dysplasia, resistant to the course of conservative treatment, it is advisable do not delay use the method of joint arthroplasty.

Остеоартроз у разі епіфізарної дисплазії (ЕД) навіть у молодому віці спричинює порушення соціальної адаптації пацієнтів, що обумовлює актуальність вивчення проблеми оптимізації їхнього ортопедичного лікування. Мета. Вивчити гістологічні особливості суглобового хряща та репаративний потенціал кісткового мозку, які визначають патогенез і впливають на тактику лікування коксартрозу у хворих із рентгенологічними ознаками ЕД. Методи. Обстежено 68 дорослих хворих на коксартроз за наявності рентгенологічних ознак ЕД. У 52 випадках виконано тотальне ендопротезування кульшових суглобів, отримано фрагменти суглобового хряща для гістологічного дослідження, кісткової тканини епіфізів — для вивчення репаративного потенціалу кісткової тканини. Результати. Встановлено, що за умов коксартрозу в пацієнтів із рентгенологічними ознаками ЕД патологічний процес розгортається на фоні глибоких порушень мікроструктурної організації матриксу суглобового хряща внаслідок змін типу організації колагенової сітки. Указані зміни обумовлюють проградієнтність перебігу захворювання та його резистентність до загальноновизнаного консервативного лікування. Виявлено значно підвищений проліферативний потенціал стромальних клітин кісткового мозку за суттєво знижену кількість колінеутворюючих одиниць фібробластів в одиниці об'єму спонгіозної тканини епіфізів, що свідчить про загрозу декомпенсації репаративних можливостей кістки. Висновки. Патогенетичними чинниками остеоартрозу у хворих із рентгенологічними ознаками ЕД є порушення організації колагену в матриксі суглобового хряща та супутні макроскопічні зміни форми епіфізів кісток нижніх кінцівок. Сьогодні не існує можливостей попередження й етіологічної терапії остеоартрозу в таких пацієнтів, при цьому є загроза декомпенсації репаративних можливостей губчастої кістки епіфізів. Доцільне застосування методу ендопротезування суглобів. Ключові слова. Коксартроз, суглобовий хрящ, репаративний потенціал кісткового мозку.

Key words. Coxarthrosis, articular cartilage, bone marrow reparative potential

Introduction

One of the consequences of genetically determined anomalies of type II collagen synthesis, which include numerous congenital anomalies of the development of epiphyses under the general term «epiphyseal dysplasias» (ED) [1–4], is the development of early osteoarthritis, accompanied by a corresponding decrease in the quality of life and an increase in the need in medical and social assistance [2, 5–10]. According to published information [1, 3, 4, 10–12], 5–29 % of the population have phenotypic signs of various types of collagenopathies with clinically expressed lesions of the musculoskeletal system, and osteoarthritis is steadily progressing on their basis. There is currently no etiological therapy for this impairment, which reduces the treatment of patients to slowing down the progression of osteoarthritis through symptomatic therapy with the timely application of radical treatment methods in order to preserve the social adaptation of patients [5, 6, 9, 10, 13–15]. But the presence of signs of epiphyseal dysplasia in patients with osteoarthritis of the joints of the limbs is quite often overlooked by practicing doctors, which disorients the prospects for the use of conservative treatment methods and leads to unjustified postponement of effective surgical intervention [12, 15–17, 18–20].

The purpose of the study: to assess histological features of the articular cartilage and the reparative potential of the bone marrow, which determine pathogenesis and influence the tactics of treatment of coxarthrosis in patients with radiological signs of epiphyseal dysplasia.

Material and methods

In accordance with the decision of the bioethics commission of the State Institution the Institute of Traumatology and Orthopedics of the National Academy of Medical Sciences of Ukraine (Protocol No. 3 of 13.09.2022), the study was performed in compliance with the norms of the Helsinki Convention of the Council of Europe on Human Rights and Biomedicine and the Laws of Ukraine. All patients included in the study signed an informed consent.

Characteristics of patients

The study involved 68 patients aged from 20 to 70 years (men — (36 ± 4), women — (37 ± 6)), who were referred to the clinic due to stage II–IV coxarthrosis (according to the classification of J. N. Kellgren, J. S. Lawrence, 1957) and who were found to have signs of epiphyseal dysplasia (REED) following additional radiological examination. In 52 cases, total endoprosthetic repair of hip and knee joints was per-

formed, which made it possible to obtain fragments of articular cartilage for histological examination and bone tissue of epiphyses to study the reparative potential of bone marrow.

Methods of the study

Fragments of articular cartilage were selected from the least loaded parts of the articular surfaces of the hip joint, namely from the peripheral part of the lower segment of the femoral head. Morphological features were studied with the help of a light microscope, and histochemical methods were used to analyze the macromolecular state of the articular cartilage matrix. For light microscopy, the material was fixed in a 10 % formalin solution, decalcified in a 5 % nitric acid solution, washed, held in alcohols of increasing concentration, in Nikiforov's solution, and embedded in celloidin. Sections with a thickness of 5–7 µm were stained with hematoxylin and eosin, as well as Van Gieson's picrofuchsin and analyzed under an AxiostarPlus light microscope. Photographs were taken using a Canon digital camera.

Histochemical reactions to collagen and glycosaminoglycans (GAGs) were used to assess the macromolecular state of the articular cartilage matrix. To study collagen types in polarized light, the sections were stripped of celloidin and stained with sirius red. During this study, the mature forms of collagen type I glow red in polarized light, collagen type II glows yellow, and collagen III glows green. Weak or absent refraction of collagen fibers reflects disorganization of molecules as a result of collagenolysis. For the analysis of sulfated GAGs (chondroitin-4- and chondroitin-6-sulfate), sections were treated with toluidine blue at pH 2.5, then studied under a Polmy-A polarizing microscope and photographed with a Canon digital camera.

The study of the osteogenic activity of bone marrow stem stromal cells (BMSSCs) was performed in 13 patients with coxarthrosis and epiphyseal dysplasia. Cloning of BMSSCs was carried out according to the method of O. Ya. Friedenstein (1973) modified by V. S. Astakhova (1982) [21]. The material for the study was cancellous bone, which was obtained from the head of the femur during endoprosthetic repair. Cloning was carried out under standard conditions for 14 days without changing the culture medium in Petri dishes at 37 °C in a gas mixture with 5 % CO₂ content in atmospheric air using lethally irradiated rabbit bone marrow cells as a feeder.

The osteogenic activity of bone marrow stem stromal cells was assessed by the total number of nucleated cells, that of stem stromal cells by the number of colony-forming units of bone marrow fibroblasts

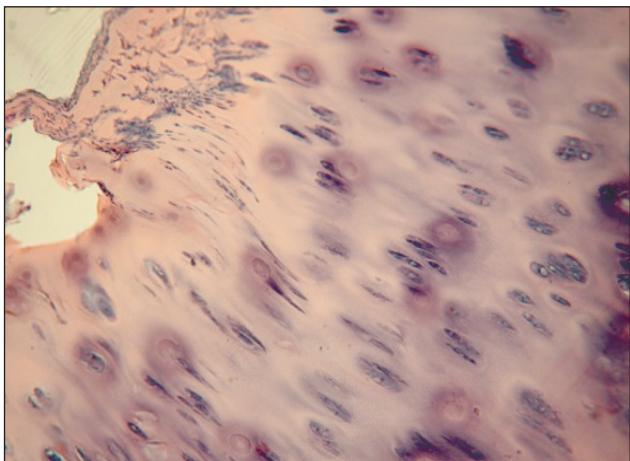


Fig. 1. Cells with a narrow elongated cytoplasm, located with the long axis perpendicular to the articular surface. Lacunae without chondrocytes. Expressed basophilia of lacunae in the deep zone of articular cartilage. H&E staining

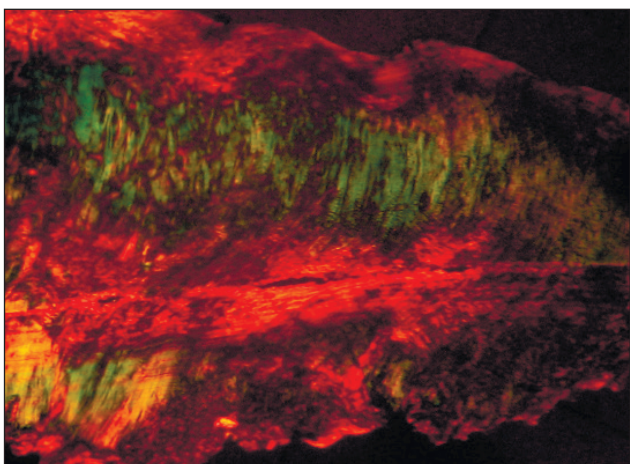
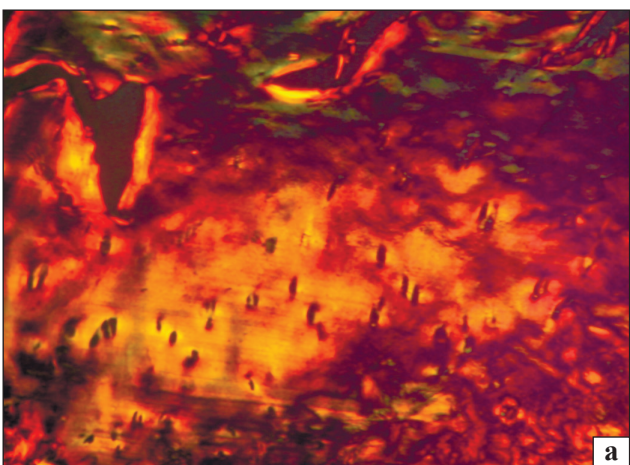


Fig. 2. Articular cartilage of the femoral head of a patient with REED in polarized light. Red color corresponds to type I collagen, green to type III, yellow to type II. Sirius red staining. x 650 magnification



(CFUf) in 1 cm³. The cloning efficiency of CFUf was determined by the formula:

$$K / N \times 10^5, \quad (1)$$

where K is the number of colonies that grew in a Petri dish; N is the number of cells planted in a Petri dish. The amount of CFUf in 1 cm³ was determined by the formula:

$$(K \times N) / (N \times V), \quad (2)$$

where K is the number of colonies that grew in a Petri dish; N is the number of cells washed from the cancellous bone sample; N is the number of planted cells; V is the volume of the cancellous bone sample.

Calculations were carried out in each experiment and on average in the group. *Statistical processing* of the obtained material was performed using the Statistica software package. The average values are given as the mean value of the indicator and the standard error of the mean is $M \pm m$. To analyze the indicators of osteogenic activity of the stem stromal cells of the bone marrow of the femoral head of patients with REED, the results of our previous studies on the evaluation of the regenerative potential of the cancellous bone of the proximal epimetaphysis of the femur in normal conditions (control group) and similar indicators of patients with idiopathic coxarthrosis with a slow course of the disease were used [22].

Results and their discussion

Determination of osteogenic activity of BMSSCs

In total, 16 cancellous bone samples were studied, from which 20 cultures of bone marrow mesenchymal stromal cells were obtained. Significant results were obtained in 13 cases, and in the remaining cultures, fungi of the genus *Aspergillus fumigatus* and *Aspergillus niger* and other bacterial flora germinated.

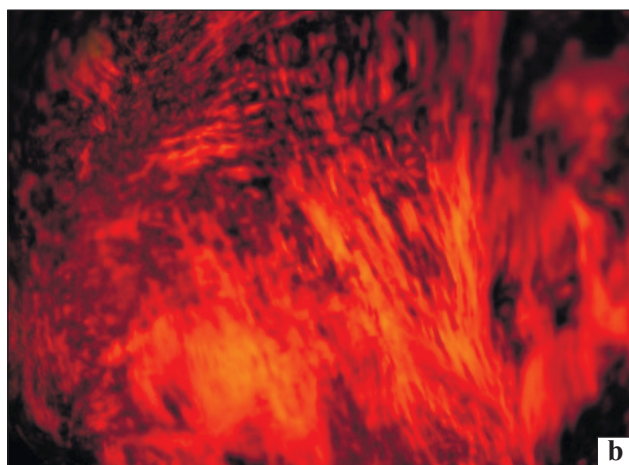


Fig. 3. Articular cartilage of the femoral head in polarized light: a) disorder of the arcade type of type II collagen organization in a patient with coxarthrosis and REED; b) orientation orderliness of collagen fibers under conditions of idiopathic coxarthrosis. Sirius red staining. x 650 magnification

Indicators of osteogenic activity of bone marrow stem stromal cells obtained from femoral heads

Group of patients	Total number of nucleated cells in 1 cm ³ × 10 ⁷	Number of CFUf in 1 cm ³ of spongiosa × 10 ⁴	Efficiency of CFUf cloning
Study (REED)	0.95 ± 0.38 n = 16	0.16 ± 0.11 n = 13	32.50 ± 22.44 n = 13
Comparison (idiopathic coxarthrosis with a slow course)	1.33 ± 1.07 n = 9	0.026 ± 0.01 n = 9	5.62 ± 4.54 n = 9
Control	13.06 ± 0.70 n = 39	1.28 ± 0.13 n = 126	10.80 ± 2.00 n = 126

The assessment showed certain differences in the indicators of osteogenic activity (cloning efficiency) in the observation groups (see Table).

Under normal conditions, an average of $(13.06 \pm 0.7) \times 10^7$ nucleated cells per 1 cm³ are counted in the head of the femur of an adult, the content of CFUf of the bone marrow per volume unit is $(1.28 \pm 0.13) \times 10^4$, and the cloning efficiency among 105 nuclear cells is (10.8 ± 2.0) . In patients with REED, the number of nucleated cells in 1 cm³ was 1.4 and 13.75 times lower than the indicators of patients with idiopathic coxarthrosis and control group, respectively. Regarding the effectiveness of bone marrow CFUf cloning, in the case of coxarthrosis in patients with REED, it was 5.8 and 3 times higher than in the comparison and control groups, respectively.

Thus, the comparative analysis of osteogenic activity indicators of BMSSCs of the femoral head shows that in patients with coxarthrosis, in the presence of REED, secondary to a significantly reduced number of CFUf per volume unit of spongiosa, their proliferative potential is significantly increased. However, clonogenic activity at such a high level cannot be maintained indefinitely, the proliferative potential and golden reserve of the BMSSCs are exhausted too quickly, and a state of decompensation of the bone's reparative capabilities occurs.

Histological studies

The study of histological sections of the articular cartilage of patients with REED under a light microscope showed the presence of both universal changes for osteoarthritis and differences in the structural organization of non-calcified cartilage. In particular, directly in the zone of calcified cartilage, there were elongated cells with elongated filamentous nuclei, located with their long axis perpendicular to the articular surface and similar in phenotype to fibroblastic diferon (Fig. 1).

A polarized light study of fragments of articular cartilage obtained during surgery in patients with coxarthrosis and REED determined a low content

of type II collagen almost to its complete disappearance and a predominance of collagen types I and III. A significant content of mature type I collagen was found not only in the surface zone of articular cartilage, but also in its larger part. Bundles of collagen fibers containing type I collagen were randomly arranged and had an oblique or vertical orientation to the surface of the cartilage (Fig. 2).

In the areas containing type II collagen fibers, their distribution in patients with REED did not correspond to the classic arcade type of organization of the collagen network in the articular cartilage matrix (Fig. 3, a). The direction of bundles of collagen fibers was markedly different from that in the articular cartilage of patients with the corresponding stage of idiopathic coxarthrosis (Fig. 3, b).

Conclusions

Patients with radiological signs of epiphyseal dysplasia are shown to have clinically significant signs of coxarthrosis at a working age, which determines the social importance of the timely application of treatment methods that can be used to maximally restore the functional capabilities of the joint.

The advanced course of coxarthrosis in the patients of the study group is associated not only with changes in the shape of the epiphyses of the bones of the lower limbs, but also with deep microstructural disturbances in the organization of the articular cartilage matrix, namely, with changes in the type of organization of the collagen network.

Under the conditions of coxarthrosis in patients with radiological signs of epiphyseal dysplasia, when choosing a management strategy, the significantly increased proliferative potential of bone marrow stromal cells should be taken into account secondary to a significantly reduced number of CFUf per unit volume of cancellous bone, which indicates a risk of decompensation of its reparative capabilities and makes it expedient to timely apply the method of endoprosthetic repair of the joint.

Conflict of interest. The authors declare no conflict of interest.

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HISTOLOGICAL FEATURES OF ARTICULAR CARTILAGE AND BONE MARROW REPARATIVE POTENTIAL UNDER CONDITIONS OF COXARTHROSIS IN PATIENTS WITH RADIOGRAPHIC SIGNS OF EPIPHYSEAL DYSPLASIA

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