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## Biochemical markers of blood serum and articular cartilage in different forms of idiopathic coxarthrosis progression

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*Deformation due to arthrosis of the hip joint affects 40 % of the total number of patients with osteoarthritis, which is from 7 to 25 % of the adult population. For the activity of the pathological process evaluation we studied biochemical changes in the metabolism of collagen, namely: glycosaminoglycans (GAG), hydroxyproline (HP) fractions, collagenase, hyaluronidase activity, etc. Objective. To determine the biochemical changes in blood serum and articular cartilage depending on the form of progression of idiopathic coxarthrosis. Methods. 23 patients were examined (8 (34.8 %) men, 15 (65.2 %) women, age 45–75 years and more) with idiopathic arthrosis of the hip joint IV stage (Kellgren–Lawrence). Progression forms of idiopathic coxarthrosis were distinguished: group I — rapid (the period from the initial to the final stage of the disease was 5 years and less), II — moderate (5–10 years), III — slow (over 10 years). Collagenase activity, HP fractions, total GAG content were determined in patients' blood serum, and collagen content was determined in the cartilage tissue of the femoral head. Results. Compared with the age reference norm in the blood serum of patients of group I activity collagenase increased to 114 %, in II and III — 122 and 135 %, respectively; the content of the free fraction of GP — up to 111, 169, 128 %, respectively; GAG concentrations — up to 110, 122, and 135 %, respectively; protein-bound HP was reduced to 60% in group II, up to 84 % — in group III. In cartilage tissue, the GAG content decreased in group III to 63 %, II — 55 %, I — 47 %; collagen — 32, 25, 24 % in accordance. Conclusions. The course of idiopathic coxarthrosis is determined by metabolic changes in the components of the organic basis of connective and cartilaginous tissues in blood serum. The most profound changes were found in the synthetic phase of HP metabolism, especially in the group with a rapid course disease. A decrease in the content of organic components of connective tissue (GAG and collagen) was determined.*

*Деформація через артроз кульшового суглоба (коксартроз, КА) вражає 40 % від загальної кількості хворих на остеоартроз, що становить від 7 до 25 % дорослого населення. У 60 % випадків КА стає причиною зниження працездатності, 11,5 % — інвалідності. Задля оцінювання активності патологічного процесу вивчають біохімічні зміни в метаболізмі колагену, а саме: глікозаміногліканів (ГАГ), фракцій гідроксипроліну (ГП), активності колагенази, гіалуронідази тощо. Мета. Визначити особливості біохімічних змін у сироватці крові та хрящовій тканині суглобового хряща залежно від форми прогресування ідіопатичного коксартрозу. Методи. Обстежено 23 пацієнти (8 (34,8 %) чоловіків, 15 (65,2 %) жінок, вік 45–75 років і більше) з ідіопатичним артрозом кульшового суглоба IV стадії за Kellgren–Lawrence. Виділено форми прогресування ідіопатичного КА: група I — швидка (термін від початкової до кінцевої стадії захворювання становив 5 років і менше), II — помірна (5–10 років), III — повільна (понад 10 років). У сироватці крові пацієнтів визначали активність колагенази, фракції ГП, сумарний вміст ГАГ, у хрящовій тканині головки стегнової кістки — вміст колагену та ГАГ. Результати. Порівняно з віковою референтною нормою в сироватці крові пацієнтів групи I активність колагенази зросла до 114 %, у II та III — 122 і 135 % відповідно; вміст вільної фракції ГП — до 111, 169, 128 % відповідно; концентрації ГАГ — до 110, 122, і 135 % відповідно; білковозв'язаний ГП виявився зниженим до 60 % у групі II, до 84 % — III. У хрящовій тканині вміст ГАГ знизився в групі III до 63 %, II — 55 %, I — 47 %; колагену — 32, 25, 24 % відповідно. Висновки. Перебіг ідіопатичного коксартрозу визначається метаболічними змінами компонентів органічної основи сполучної та хрящової тканин у сироватці крові пацієнтів. Найглибші зміни виявлено в синтетичній фазі метаболізму ГП, особливо в групі зі швидким перебігом захворювання. Визначено зниження вмісту органічних компонентів сполучної тканини (ГАГ та колагену). Ключові слова. Коксартроз, стадії прогресування, біохімічні зміни, сироватка крові, суглобовий хрящ.*

**Keywords.** Coxarthrosis, stages of progression, biochemical changes, blood serum, articular cartilage

## Introduction

Deformation due to arthrosis of the hip joint (coxarthrosis, CA) accounts for 40 % of the total number of patients with osteoarthritis and affects from 7 to 25 % of the adult population. In 60 % of cases, CA becomes the cause of reduced work capacity, with disability in 11.5 % [1–4]. This indicates the high medical and social significance of the disease, which not only significantly worsens the patient's quality of life, but also leads to large social and economic costs for society [5, 6].

CA is a polyetiological disease, the occurrence and development of which are associated with a number of genetic, endogenous (hormonal imbalance, immune disorders, etc.) and exogenous factors (trauma, overload). Most experts believe that cartilaginous tissue is primarily affected by CA [7–9]. Development of this disease is associated with violations of the structure and function of the cartilage matrix and its metabolism [10, 11]. The main pathological manifestation of CA is the destruction of articular cartilage, the most important function of which is adaptation of the joint to mechanical load and ensuring free movement of articular surfaces [12].

CA is conditionally divided into primary and secondary. The primary one occurs in previously healthy cartilage under the influence of increased load, in the case of the secondary one, degradation of previously changed articular cartilage occurs under the influence of even a normal load [13]. According to the classification developed by the American College of Rheumatology, idiopathic (localized and generalized) CA belongs to primary, and forms that occur against the background or after suffered diseases or injuries (post-traumatic, dysplastic, dysmetabolic) belong to secondary [13].

The presented numerous studies mainly reflect the general reaction of the body to the presence of a pathological process and are specific for this disorder. Pathobiochemical processes that disrupt the entire system of connective tissue are generally accompanied by significant changes in the metabolism of collagen, as well as glycosaminoglycans (GAG). In recent years, along with GAG, hydroxyproline (HP) has attracted attention as the most objective marker of metabolic processes in connective tissue, which determines its biomechanical and physiological properties. Since it is present in the body only in the composition of fibrillar proteins of connective tissue, mostly collagen, HP detected in the blood and urine of patients has a metabolic relationship with these proteins [14]. In addition, there can also be a re-

duction of HP molecules, which acquire the ability to leave the cartilage matrix and, changed and small, to absorb water without the possibility of its retention. Excess water, absorbed by collagen, swells and breaks up into separate fibers, which leads to a decrease in its resistance [12]. The study of the content of the amino acid fractions of HP is used to assess the depth of collagen metabolic disorders, the severity of the pathological process, and to monitor the dynamics of the course. A special place among connective tissue metabolites is occupied by GAGs, which fill the intercellular space in the structure of this tissue [15]. Changes in the activity of such enzymes as collagenase, hyaluronidase, involved in the metabolism of collagen and GAG, are now considered the most sensitive, specific and early indicators of the pathological process.

*Purpose of the study:* to determine the features of biochemical changes in blood serum and cartilage tissue of articular cartilage depending on the form of progression of idiopathic coxarthrosis.

## Material and methods

The clinical research plan was approved by the Bioethics Committee of the State Establishment “Institute of Traumatology and Orthopedics of the National Academy of Medical Sciences of Ukraine” (Protocol No. 275 dated 08.05.2021). Voluntary consent was obtained from patients who became the object of research.

### *Patients*

The study involved examination of 23 patients (8 (34.8 %) men, 15 (65.2 %) women) with idiopathic arthrosis of the hip joint stage IV according to J. H. Kellgren and J. S. Lawrence [16]. By age, the patients were distributed as follows: 45–49 years old — 6 (26.1 %), 2 men and 4 women; 60–74 — 13 (56.5 %), 4 and 9 people, respectively; 75 and over — 4 (17.4 %), 2 people each. The following forms of idiopathic CA have been distinguished: rapidly progressive (the period from the initial to the final stage of the disease was 5 years or less), moderately progressive (over 5 to 10 years) and slowly progressive (over 10 years) [10, 17–19].

### *Biochemical research*

In order to assess the activity of the pathological process, metabolic changes in the organic basis of bone and cartilage tissues were studied. To solve these tasks, the following biochemical indicators were determined in the blood serum of patients: collagenase activity — according to the method of S. Lindy, J. Halme [20], using as a substrate collagen from the company Sigma; HP fractions were

isolated according to the method of S. Frey [21], and HP in them according to H. J. Stegemann [22]; the total GAG content according to S. A. Klyatskin and R. I. Lifshits [15].

To determine the condition of the articular cartilage of patients, a sample of the cartilage tissue of the femoral head, obtained during surgical interventions, was placed in an alcohol-ether mixture in a ratio of 3:1 for 24 hours, kept in ether for 5–6 hours, and then in a thermostat for 2–3 hours. Immediately before analysis, the tissue was frozen in liquid nitrogen and ground into a powder. The metabolism of collagen and GAG was studied in cartilage tissue [9, 14, 15, 23]. The results obtained during the study were subjected to statistical processing in comparison with the age reference norm [24].

**Results and their discussion**

The analysis of biochemical indicators obtained as a result of the study of the blood serum of patients with idiopathic CA showed that in the group with a rapid form of progression of the pathological process, collagenase activity increased to 114 % compared to the values in the control, and in patients with a moderate and slow course to 122 and 135 % respectively. In absolute terms, it was

equal to  $(3.57 \pm 0.10)$   $\mu\text{mol}/\text{l}\cdot\text{h}$  in the fast form of the course, in moderate and slow —  $(3.83 \pm 0.11)$  and  $(4.32 \pm 0.35)$   $\mu\text{mol}/\text{l}\cdot\text{h}$ , respectively (Table).

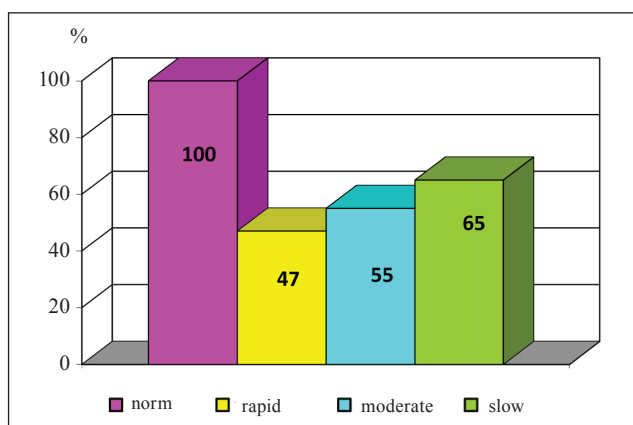
Along with an increase in the activity of collagenase, an enzyme involved in the catabolic phase of collagen metabolism, an increase in the content of the free fraction of HP, a biochemical marker of collagen breakdown, was established. In particular, in the group with rapid progression of idiopathic CA — 111 %, moderate — 169 %, slow — 128 % compared to the control group. At the same time, the biochemical marker of the synthetic phase of collagen metabolism — protein-bound HP turned out to be reduced compared to the level of the control group and was 60 % in patients with a moderate course of the disease, 84 % — with a slow course (Table).

Thus, as a result of the analysis of the studied indicators that reflect the activity of enzymes in the blood serum of patients, and the content of biochemical markers of the synthesis of collagen as the main protein of bone and cartilage tissues, their most pronounced deviation was established in the synthetic phase of the metabolism of this protein, regardless of the form of the course of CA, especially in patients with a rapid and moderate course of the disease.

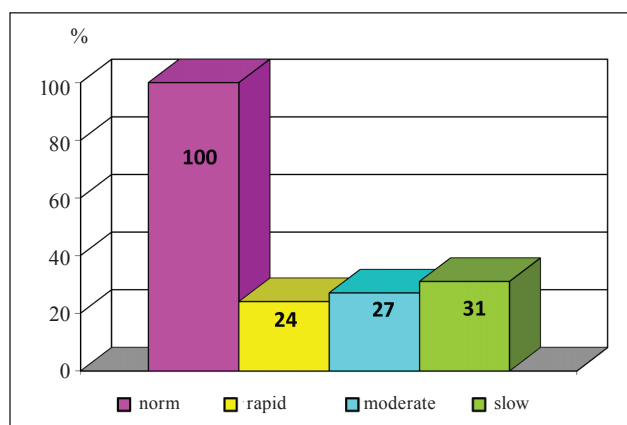
Table

**Biochemical indicators of blood serum of patients with idiopathic CA**

Indicator	Norm (n = 8)	Form of idiopathic CA course		
		rapid (n = 8)	moderate (n = 8)	slow (n = 7)
Collagenase, $\mu\text{mol}/\text{l}\cdot\text{h}$	$3.140 \pm 0.040$	$4.470 \pm 0.220$	$3.920 \pm 0.110$	$3.590 \pm 0.100$
HP, $\mu\text{mol}/\text{l}$ :				
– free	$5.750 \pm 0.210$	$9.550 \pm 0.300$	$6.470 \pm 0.200$	$7.330 \pm 0.210$
– protein bound	$11.900 \pm 0.290$	$7.120 \pm 0.180$	$8.080 \pm 0.130$	$10.170 \pm 0.400$
GAG, g/l	$0.031 \pm 0.003$	$0.034 \pm 0.001$	$0.038 \pm 0.001$	$0.042 \pm 0.001$



**Fig. 1.** GAG content in cartilage tissue in patients depending on the forms of progression of idiopathic coxarthrosis



**Fig. 2.** Collagen content in cartilage tissue depending on the forms of progression of idiopathic coxarthrosis

When determining the concentration of GAG in the blood serum of patients, it was also found to increase: in the group with a rapid course of CA up to 110% compared to the control group (in absolute terms —  $(0.034 \pm 0.001)$  g/l); with moderate and slow-up to 122 and 135 %, respectively (Table).

Similar changes were determined as a result of studying the components of the cartilage matrix. The analysis of the obtained GAG values in the cartilage tissue of patients with idiopathic CA with a slow form of the disease showed their decrease to 63 % (absolute values —  $(2.30 \pm 0.08)$  µg/mg against the reference norm  $(3.64 \pm 0.12)$  µg/mg). Even more pronounced changes were found in the GAG content in the group with a moderate course of the disease —  $(2.00 \pm 0.16)$  µg/mg, rapid —  $(1.72 \pm 0.10)$  µg/mg, which was 55 and 47 %, respectively, from the indicators of the control group (Fig. 1).

Regarding the main protein of connective tissue (collagen), a more pronounced deviation from the norm was recorded. In patients with idiopathic CA of a slow form, the progression was reduced to 32 %, and in absolute terms — to  $(1.57 \pm 0.04)$  mg/g against  $(4.90 \pm 0.20)$  mg/g in the norm. The most pronounced decrease in collagen content was observed in moderate and rapid forms of disease progression: up to 25 and 24 % compared to the control, respectively, in absolute numbers —  $(1.24 \pm 0.10)$  mg/g and  $(1.19 \pm 0.06)$  mg/g, respectively (Fig. 2).

The results obtained during the study of cartilage tissue in patients with idiopathic CA indicate a decrease in the content of organic components of connective tissue (Fig. 1, 2). The course of the disease is determined by metabolic changes in the components of the organic basis of cartilage tissue. In patients with a rapid course of the pathological process, violations were more pronounced in GAG indicators.

Thus, the obtained features of biochemical changes in blood serum and cartilage tissue of articular cartilage of patients with various forms of progression of idiopathic CA allow us to assume that there is a violation of metabolic processes — synthesis of collagen and GAG, and these changes lead to faster development of the disease and deepening of the pathological process.

## Conclusions

The course of idiopathic coxarthrosis is determined by metabolic changes in the components of the organic basis of connective and cartilaginous tissues in the blood serum of patients. The most profound changes were found in the synthetic phase of HP metabolism, especially in the group with

a rapid course of the disease. A decrease in the content of organic components of connective tissue (GAG and collagen) was determined.

Metabolic changes in the components of the organic basis of the cartilage tissue of the femoral head have been established. In patients with a rapid course of the pathological process, the disturbances are more pronounced, especially the GAG indicators.

Certain regularities contribute to a better understanding of the pathogenesis of CA, which should be taken into account during diagnostic and treatment measures in patients with this severe orthopedic disorder.

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

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## BIOCHEMICAL MARKERS OF BLOOD SERUM AND ARTICULAR CARTILAGE IN DIFFERENT FORMS OF IDIOPATHIC COXARTHROSIS PROGRESSION

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