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Biochemistry

Biochemical Changes of the Connective Tissue Metabolism in Patients with Knee Osteoarthrosis

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The study of metabolic changes in the organic matrix of bone-cartilage tissue at different stages of the disease may help us deepen the knowledge of its pathogenesis and treatment methods. The same studies enable us to determine the severity of the pathologic process and the efficiency of treatment. All the mentioned confirms the necessity to study metabolic changes in the organic matrix of cartilage tissue to understand the mechanism of the pathology development. The task is to study the changes in collagen and GAG metabolism of patients with knee osteoarthrosis depending on the stage of the disease. We have examined 35 patients with knee osteoarthrosis (the patients of the SI "The Institute of Traumatology and Orthopedics", NAMS of Ukraine, and "Feofaniia" clinical hospital of the State Management of Affairs), among them 13 persons with the 2nd stage, 10 – with the 3rd stage, and 12 – with the 4th stage of the disease. We took measures to ensure the patients' security and health, respect to their rights, human dignity, moral and esthetic norms according to the principles of the Helsinki Declaration the protection of human rights, Council of Europe Convention for the Protection of Human Rights and Fundamental Freedoms and biomedicine, and the respective laws of Ukraine. In the blood serum of the patients, we determined their collagenase, hydroxyproline fraction, glycosaminglycanes, and alkaline phosphatase activity. The data obtained (the activity of collagenase, hydroxyproline fraction (HP), glycosaminglycanes (GAG) and alkaline phosphatase (ALP)) within the examination of the knee osteoarthrosis patients enable us to consider their values as biochemical markers for the pathological process and treatment efficiency. *© 2021 Bull. Georg. Natl. Acad. Sci.*

Osteoarthrosis, collagenase, hydroxyproline fraction, glycosaminoglycans, alkaline phosphatase

Osteoarthrosis is a chronic degenerative disease of synovial joints with a background in initial or secondary joint cartilage degeneration with the further development of reactive synovitis, changes in bones' subchondral portions, joint deformities [1-3]. It is one of the most frequent articular degenerative-dystrophic diseases, found in 6.4 to 12% of the world population [4].

Literature confirms that the COMP (non-collagen protein of joint cartilage matrix) level

gives essential information on metabolic changes in the cartilage matrix in joint diseases [5,6]. Many publications reflect the general inflammatory response to the pathological process and are not specific to this disorder.

Pathologic biochemical processes impairing the system of connective tissue as a whole are associated with massive changes in the metabolism of collagen and glycosaminglycanes (GAG). The most objective clinical indicators of metabolic processes in the connective tissue that attract attention in recent years are hydroxyproline (HP) and GAG, determining biomechanical and physiological characteristics of the connective tissue. HP is known to exist in a body within the fibrous proteins of the connective tissue, foremost collagen. Obviously, HP discovered in the blood and urine of patients metabolically relates to these proteins [7].

The study of changes occurring in the connective tissue at different stages may extend our knowledge of the pathogenesis of this disease and the methods of management. The same studies enable us determining the pathologic process severity and the efficiency of the treatment.

Typical of osteoarthrosis is the inflammation development in the articular cartilage, followed by metabolic disorders in the organic matrix represented by one of the connective tissue types [8].

The content of the HP amino acid fractions serves to evaluate the extent of metabolic impairment of the connective tissue basic protein – collagen; assess the severity of the pathological process, observe the process's dynamics. GAG plays a specific role among the connective tissue metabolites, as it fills the intercellular space in this tissue's structure [7].

Changes in the activity of collagenase that takes part in the collagen's metabolism are currently considered the most sensitive, specific, and early sign of the inflammation process.

Thus, determination of HP, GAG fractions, and the activity of this enzyme, the essential participant of this protein's metabolism – collagenase in the blood serum of patients with osteoarthrosis, enables us to state the extent of metabolic processes impairment on the organic matrix of the bone.

All above confirm the necessity to study metabolic disorders in the organic matrix of the cartilage to understand the mechanism of the pathological process development.

TASK is to study changes in the metabolism of collagen and GAG in patients with knee osteoarthrosis depending on the stage of the disease.

Materials and Methods

The study involves 35 patients with knee osteoarthrosis, (the patients of the SI “The Institute of Traumatology and Orthopedics”, NAMS of Ukraine, and “Feofaniia” clinical hospital of the State Management of Affairs) namely 13 persons with the 2nd stage, 10 with the 3rd stage, and 12 with the 4th stage of the disease. Analysis of the blood serum of these patients studied the following biochemical values: collagenase activity by Lindy S., Halme J. [9], HP fractions according to Frey S. [10], hydroxyproline by Stegemann H.J. [11], GAG by Kliatskin S.A. and Lifshyts R.I. [12], alkaline phosphatase (ALP) – on Cobas-311 biochemical analyzer with the set of reagents (by Roshe diagnostics, Germany).

The obtained biochemical data underwent statistical processing.

Results and Discussion

Analysis of biochemical values of the blood serum in patients with the 2nd stage of osteoarthrosis discovered the increased activity of one key enzyme in the metabolism of the connective tissue basic protein collagen – collagenase, reaching 5.23±0.23mmol/l*h (166%). In the same patients, we revealed the increased concentration of HP free fraction – 6.53±0.12mmol/l (114%) – the bio-chemical marker of bone tissue resorption followed by the decreased content of protein-connected HP

Table. Biochemical values of the blood serum in patients with knee osteoarthrosis

Stage	Collagenase, mmol/l*h	HP fractures, mmol/l		GAG, g/l	ALP, u/l	
		free	Protein-connected		total	bone
2nd	5.23±0.23	6.53±0.12	8.39±0.13	0.072±0.002	70.63±0.75	25.67±0.50
3rd	6.24±0.14	7.28±0.13	7.74±0.21	0.099±0.003	79.62±0.82	27.93±0.48
4th	8.50±0.12	9.75±0.13	7.50±0.13	0.104±0.003	94.64±0.98	34.45±0.42
Normal	3.14±0.04	5.75±0.21	11.90±0.29	0.031±0.003	65.60±5.20	26.20±2.10

8.39±0.13 (70%) – the biochemical indicator of collagen protein synthesis (Table, Fig.1).

The content of GAG that determines physiological and biomechanical features of the cartilage reaches 0.072±0.002 g/l, compared to the normal one.

The activity of ALF, the osteoblastic activity marker, grows to 108%, compared to the normal one (Table, Fig.1).

Thus, patients with the 2nd stage of knee osteoarthrosis have their metabolic processes impaired as in the main connective tissue protein – collagen, as in GAG exchange. The biochemical results reflect destructive changes in the tissues of these patients' joints.

The patients with the 3rd stage of the disease have more changes in the collagenase activity, reaching 6.24±0.14 mmol/l*h. The values of the collagen-exchange metabolites reflect the high concentration of the HP free fraction (127%) – the biochemical marker of collagen protein catabolic phase with the low concentration of the protein-

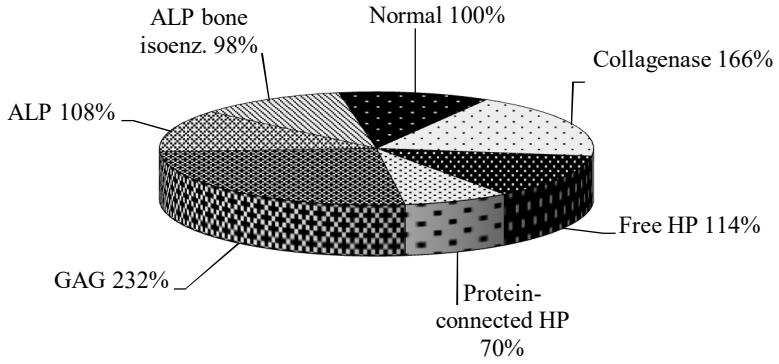

Fig. 1. Biochemical values of the blood serum in patients with the 2nd stage of knee OA.

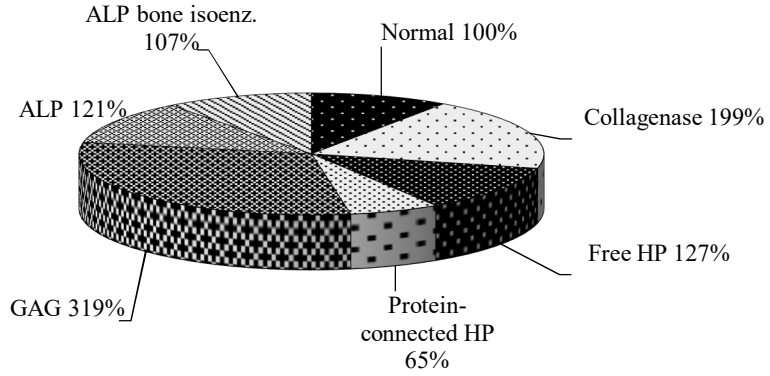

Fig. 2. Biochemical values of the blood serum in patients with the 3rd stage of knee OA.

connected HP (65%), indicating the synthesis of the said protein (Table, Fig.2).

Especially expressed, these changes appear in GAG metabolism, reaching 0.099±0.003 g/l (319%), more than three times exceeding the normal. The activity of ALP is 121%, in absolute values – 79.62±0.82 U/l, confirming the high osteoblastic activity (Table, Fig.2).

The collagenase activity in patients with the 4th stage of the disease has grown more and reached 270% compared to the norm (8.50±0.12 mmol/l*h) (Table, Fig.3). The concentration of free HP fractures was 9.75±0.13 mmol/l (170%), and the content of the protein-connected HP – 7.50±0.13 mmol/l (63%).

The values of GAG exchange showed even more destructive changes in their metabolism and achieved 0.104±0.003 g/l (335%). The values reflecting the ALP confirm the growth in the osteoblastic activity, approved by the levels of its bone isoenzyme (Table, Fig.3).

The comparison of the result in patients with different stages of osteoarthrosis shows that patients with the 4th stage of the disease have the most expressed divergence as in collagen exchange, as in GAG metabolism, compared with the same on the 2nd and the 3rd stages of the pathological process development. The collagenase activity in patients with the 2nd and the 3rd stages of the disease exceeds the norm almost twice. At the 4th stage, this enzyme almost three times exceeds its normal value.

Similar changes appear in the total content of GAG. On the 2nd stage of the disease, the GAG content of GAG more than twice exceeds the norm, on the 3rd stage – 3 times, and on the 4th stage – more than 3 times.

With the progression of osteoarthrosis, typical are the rise in GAG concentration and the increase in the free HP, followed by the growth in the collagenase activity.

Thus, metabolic changes in the organic matrix of the cartilage are accompanied by destructive disorders, leading to the pathologic process development. The higher is the stage of the disease, the more severe the metabolic disorders in the organic matrix of the cartilage are.

Conclusions

Analyzing the data obtained within the examination of the knee osteoarthrosis patients, reflecting metabolic changes in the bone-cartilage matrix (collagenase, hydroxyproline fraction, glycosaminglycanes, and alkaline phosphatase activity), we can recognize to have solid grounds to treat them as the biochemical markers for the pathologic process and efficiency of the therapy in this category of patients.

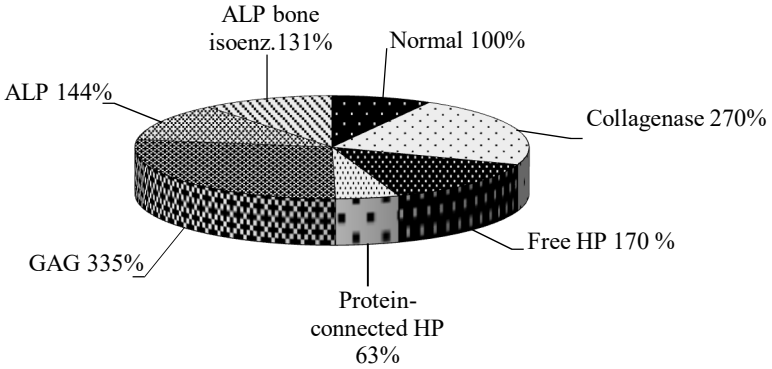

Fig. 3. Biochemical values of the blood serum in patients with the 4th stage of knee OA.

ბიოქიმია

შემაერთებელი ქსოვილის მეტაბოლიზმის ბიოქიმიური ცვლილებები მუხლის ოსტეოართროზით დაავადებულ პაციენტებში

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*უკრაინის სამედიცინო მეცნიერებათა ეროვნული აკადემია, სი ტრავმატოლოგიისა და ორთოპედიის ინსტიტუტი, უკრაინა
**ფეოფანიის“ სახელმწიფო საქმეთა მართვის კლინიკური საავადმყოფო

(წარმოდგენილია აკადემიის წევრის ნ. მითაგვარიას მიერ)

დაავადების სხვადასხვა სტადიაზე ძვალ-ხრტილოვანი ქსოვილის ორგანულ მატრიქსში მეტაბოლური ცვლილებების შესწავლა შესაძლებელია დაგვეხმაროს მისი პათოგენეზისა და მკურნალობის მეთოდების ცოდნის გაღრმავებაში. ეს კვლევა საშუალებას გვაძლევს დავადგინოთ პათოლოგიური პროცესის სიმწვავე და მკურნალობის ეფექტურობა. ყოველივე ზემოაღნიშნული ადასტურებს ხრტილოვანი ქსოვილის ორგანულ მატრიქსში მეტაბოლური ცვლილებების შესწავლის აუცილებლობას პათოლოგიის განვითარების მექანიზმის გასარკვევად. ჩვენი ამოცანაა მუხლის ოსტეოართროზით დაავადებულ პაციენტებში კოლაგენისა და გლიკოზამინოგლიკანის (GAG) მეტაბოლიზმის ცვლილებების შესწავლა, დაავადების სტადიიდან გამომდინარე. ჩვენ გამოვიკვლიეთ მუხლის ოსტეოართროზით დაავადებული 35 პაციენტი (პაციენტები შემდეგი კლინიკებიდან: სი ტრავმატოლოგიისა და ორთოპედიის ინსტიტუტი, უკრაინის სამედიცინო მეცნიერებათა ეროვნული აკადემია და ფეოფანიის სახელმწიფო საქმეთა მართვის კლინიკური საავადმყოფო); აქედან 13 პაციენტი იყო დაავადების მეორე სტადიაზე, 10 – მესამე და 12 – მეოთხე სტადიაზე. ჩვენ მივიღეთ ზომები პაციენტების უსაფრთხოებისა და ჯანმრთელობის უზრუნველსაყოფად, მათი უფლებების, ადამიანური ღირსების, მორალური და ესთეტიკური ნორმების პატივისცემისთვის, ადამიანის უფლებების დასაცავად ჰელსინკის დეკლარაციის, ევროპის საბჭოს კონვენციისა და ძირითადი თავისუფლებებისა და ბიომედიცინის პრინციპებისა და უკრაინის შესაბამისი კანონების თანახმად. პაციენტების სისხლის შრატში დავადგინეთ მათი კოლაგენაზის, ჰიდროქსიპროლინის ფრაქციის, გლიკოზამინგლიკანებისა და ტუტე ფოსფატაზას მოქმედების ინტენსიურობა. მუხლის ოსტეოართროზის მქონე პაციენტთა გამოკვლევის შედეგად მიღებული მონაცემები (კოლაგენაზის, ჰიდროქსიპროლინის ფრაქციის, გლიკოზამინგლიკანებისა და ტუტე ფოსფატაზის მოქმედების ინტენსიურობა) გვაძლევს შესაძლებლობას განვიხილოთ მათი მნიშვნელობები, როგორც ბიოქიმიური მარკერები პათოლოგიური პროცესისა და მკურნალობის ეფექტურობისთვის.

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