

PRACA ORYGINALNA
ORIGINAL ARTICLE**DYNAMICS OF BIOCHEMICAL MARKERS OF CONNECTIVE TISSUE METABOLISM IN PATIENTS WITH KNEE OSTEOARTHRITIS DURING CONSERVATIVE TREATMENT WITH LASER THERAPY****Kyrylo V. Makolinet¹, Vasyl I. Makolinet², Dmytro V. Morozenko^{1,2}, Kateryna V. Gliebova², Svitlana I. Danylchenko³**¹SYTENKO INSTITUTE SPINE AND JOINT PATHOLOGY, KHARKIV, UKRAINE²NATIONAL UNIVERSITY OF PHARMACY, KHARKIV, UKRAINE³PETRO MOHYLA BLACK SEA NATIONAL UNIVERSITY, MYKOLAIV, UKRAINE**ABSTRACT**

Introduction: The biochemical markers of the connective tissue metabolism in blood serum may characterize the disturbances in inflammatory, destructive processes, and also serve the criteria for diagnostic evaluation of the state of cartilage and bone tissue of the joints in knee osteoarthritis to control the effectiveness of conservative treatment.

The aim: determination of biochemical markers of connective tissue metabolism in the blood of patients with the 1st and 2nd stages of knee osteoarthritis in order to prove the effectiveness of the complex conservative treatment of patients with the use of low-intensity infrared laser radiation.

Materials and methods: During the study we examined 80 patients with the 1st and 2nd stages of knee osteoarthritis according to the Kellgren and Lawrence classification. Some patients were prescribed diclofenac sodium and glucosamine hydrochloride, others were prescribed an additional course of laser therapy.

Results: At the end of the treatment the group of patients with knee osteoarthritis who were treated with the use of laser therapy, diclofenac sodium and glucosamine hydrochloride, showed a decrease in the content of the following blood serum components: glycoproteins (by 28.9 %), sialic acids (by 28.8 %), chondroitinsulfates (by 91.3 % due to chondroitin-6-sulfate, activity of alkaline phosphatase (by 36.1 %) compared with the indicators before treatment.

Conclusions: a more significant decrease in the inflammation and destruction of the cartilage tissue of the affected joints was observed during knee osteoarthritis treatment with the use of laser therapy than without it.

KEY WORDS: knee osteoarthritis, patients, laser therapy, glucosamine hydrochloride, sodium diclofenac

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INTRODUCTION

Modern views to connective tissue in the human body do not characterize it as a component of organs and systems, but present it as an integrative buffer environment. At present, it is impossible to consider connective tissue elements of joints as separate components of any pathological process, especially at knee osteoarthritis [1]. All cellular and fibrous elements of the matrix connective tissue are surrounded by gelatinous substance, which, on the one hand, participates in their integration; on the other hand, it prevents their convergence and aggregation. This substance provides metabolic processes between the blood and cells that are located outside the vascular bed. In this regard, the substance is defined as the integrative buffer metabolic environment of the connective tissue, which is known in the literature as the main substance. The degree of tissue concentration and density depends on its localization. Thus, this substance has a liquid jelly-like consistency in the intercellular space of loose unformed connective tissue, and in the hyaline cartilage tissue, it determines the elastic properties of the cartilage, which enable it to function as a shock reducer. The main substance is a multicomponent system. Its main component is glycoconjugates (glycopro-

teins and proteoglycans), as well as water [2, 3]. Proteoglycans create the structural basis of the gel, and water is a solvent for substances that are present in it, and is involved in depreciation. This water contains inorganic ions, blood proteins and urea, products of metabolism of parenchymal, myeloid and connective tissue cells, as well as products of their synthesis: soluble forms of fibrous proteins, proteoglycans, glycoproteins and complexes formed by them [4, 5]. It is obvious that the biochemical markers determining any components of glycoconjugates in biological fluids (blood, urine) can indirectly characterize metabolic disorders in inflammatory and destructive processes. They can also become the criteria for diagnostic evaluation of the state of connective tissue elements of the body, in particular, cartilage and bone tissue joints in knee osteoarthritis both for evaluating the course of the disease and determination of the leading parts of its pathogenesis, and for monitoring the effectiveness of conservative treatment [6].

THE AIM

The aim of the study was to determine the biochemical markers of connective tissue metabolism in the blood of

patients with the 1st and 2nd stages of knee osteoarthritis in order to prove the effectiveness of complex conservative treatment of patients with the use of low-intensity infrared laser radiation.

MATERIALS AND METHODS

The research was carried out on the basis of the consultative-polyclinic department, departments of conservative treatment and rehabilitation, laboratory diagnostics and immunology of the Sytenko Institute of Spine and Joint Pathology, National Academy of Medical Sciences of Ukraine. The clinical study was based on the observation of 80 patients with knee osteoarthritis of the 1st and 2nd stages according to the classification of Kellgren and Lawrence [7]. The duration of the disease varied from 2 weeks to 10 years. Additionally, we involved 30 volunteers, they made up a control group of practically healthy people, aged from 25 to 63 (mean age 38.6 ± 1.8 years).

According to anamnestic data, 14 patients (17.5 %) indicated the knee injury as a possible trigger factor in the development of knee osteoarthritis, 28 patients (35%) reported chronic overload of the lower extremities, although 38 patients (47.5 %) failed to identify the causative factor for the development of knee osteoarthritis. Taking into account the nascence of the first symptoms of the disease, the patients were distributed as follows: 15 (18.8%) of them suffered from knee osteoarthritis up to 1 month, 31 (38.8%) patient had it from 1 to 12 months, 28 (35%) patients suffered from knee osteoarthritis from 1 to 5 years, and 5 (6.6%) patients had it from 5 to 10 years.

In the process of treatment all patients were divided into 2 groups. The first group consisted of 40 patients, 16 men and 24 women. The age of the patients was from 18 to 67, and the average age was 38.2 ± 15.7 . This group of patients was treated with the use of diclofenac sodium and glucosamine hydrochloride. The second group also included 40 patients, among whom there were 15 men and 25 women. The age of patients was from 19 to 63, and the average age was 40.7 ± 16.2 years. Besides diclofenac sodium and glucosamine hydrochloride this group patients were prescribed a course of laser therapy. These patients took medications twice a day. The control test was administered to patients after 30 days from the treatment beginning. Laser therapy was performed using the CM-3 (Nizhyn Laboratories of Scanning Devices) device, which generates infrared laser radiation with a wavelength of 0.8-0.9 microns, with an output of 1.5 mW. At irradiation of knee joints, we used the scanning beam in the form of a grid 60 cm² in size with 3-4 fields. The time of one procedure did not exceed 25-30 minutes, and the dose was 0.3 J. The course of treatment consisted of 10 procedures. At the end of treatment, we performed the control clinical examination and determined the content of biochemical markers in blood serum. The research plan was approved by the Bioethics Committee of "Sytenko Institute of Spine and Joint Pathology", Protocols № 81 of December 20, 2010.

The activity of alkaline phosphatase in the patients' blood serum was determined by the kinetic method, glycopro-

teins were assessed by Steinberg-Dotsenko method, sialic acids were measured by Hess method, chondroitin sulfates were assessed by Nemeth-Csoka method in L. I. Slutskii modification, fraction of glycosaminoglycans (GAG) were determined using M. R. Stern et al method [8, 9]. The statistical analysis of the data was carried out using software packages Microsoft Excel XP and Statsoft Statistica 10.0. Comparison of patient groups was performed according to Student's parametric criterion and Wilcoxon's non-parametric criterion [10].

RESULTS AND DISCUSSION

The collapse of collagen and proteoglycans in the cartilage of the knee joints in knee osteoarthritis is reflected in such biochemical markers as glycoproteins, chondroitin sulfates, and glycosaminoglycans fractions. This fact was confirmed during the experimental study on rats [11]. According to the results of biochemical study of blood in patients with the 1st and 2nd stages of knee osteoarthritis, the content of glycoproteins was increased by 55%, sialic acids was increased by 54.7% compared with these indicators in clinically healthy subjects. Increasing their concentration in blood indicates the presence of inflammatory process in the body, apparently due to inflammatory-destructive disorders in the joints during knee osteoarthritis. Glycoproteins of cartilage tissue perform the following functions: maintain the connection of chondrocytes with the matrix and stabilize its structure, facilitate the attachment of chondrocytes to the matrix, bind calcium, participate in the formation of joints and endochondral ossification, bind to collagen of the 2nd type and proteoglycans, strengthen connections between aggrecan and hyaluronic acid. Cartilage glycoproteins also participate in the differentiation and stabilization of the chondrocytes phenotype, bind to proteoglycans, and can be expressed in osteoarthritis getting into the bloodstream. The content of chondroitin sulfates in patients with early stages of knee osteoarthritis was increased by 3.3 times due to the glycosaminoglycans fraction increase (chondroitin-6-sulfate) compared with clinically healthy people. The activity of alkaline phosphatase increased by 94.5% compared with clinically healthy people, which can be obviously explained by increased activity of bone tissue osteoblasts. All of the above-mentioned changes are due to inflammation and catabolism of proteoglycans of joints cartilage tissue in knee osteoarthritis (Table I).

In the study of glycoprotein content in blood serum of patients with knee osteoarthritis who were treated without laser therapy, we observed a decrease of this indicator by only 17.4% compared with the same indicator before treatment. The content of sialic acids in serum decreased by 18.6%, chondroitin sulfates lessened by 25.3%, and alkaline phosphatase activity dropped by 21.6% compared with the pre-treatment rates. Such a dynamics of biochemical markers data indicates a decrease in the intensity of the inflammatory process in joints during knee osteoarthritis. But it should be noted that the content of glycoproteins and

Table I. Biochemical markers will be connective tissue in serum of blood and urine in patients with early stages of knee osteoarthritis (M±m)

Biochemical markers	Control group, n=30	Patients with osteoarthritis, n=80
Glycoproteins, g/l	0.60±0.06	0.93±0.06 ***
Sialic acids, mmol/l	1.92±0.15	2.97±0.07 ***
Chondroitin sulfates, g/l	0.078±0.003	0.256±0.015 ***
Total GAG, g/l	0.123±0.009	0.120±0.003
I fraction GAG, g/l	0.060±0.003	0.084±0.002 ***
II fraction GAG, g/l	0.036±0.004	0.022±0.002 ***
III fraction GAG, g/l	0.027±0.003	0.014±0.002 **
Alkaline phosphatase, U/L	4.00±0.04	7.78±0.17 ***

Notes: ** – $p < 0.01$; *** – $p < 0.001$ compared with the control group

Table II. Dynamics of biochemical markers of connective tissue in patients with knee osteoarthritis during treatment without the use of laser therapy (Me, 25%–75%)

Biochemical markers	Control group, n=30	During the treatment, n=40	
		Before treatment	After treatment
Glycoproteins, g/l	0.60 0.48 – 0.72	0.92 0.88 – 0.93	0.76 *** 0.73 – 0.78
Sialic acids, mmol/l	1.92 1.61 – 2.23	2.90 2.80 – 3.00	2.36 *** 2.28 – 2.45
Chondroitin sulfates, g/l	0.078 0.072 – 0.084	0.245 0.231 – 0.265	0.183 *** 0.174 – 0.204
Total GAG, g/l	0.123 0.105 – 0.141	0.129 0.121 – 0.140	0.120 0.113 – 0.130
I fraction GAG, g/l	0.060 0.054 – 0.066	0.078 0.074 – 0.085	0.069 * 0.064 – 0.074
II fraction GAG, g/l	0.036 0.028 – 0.044	0.029 0.028 – 0.032	0.026 * 0.024 – 0.028
III fraction GAG, g/l	0.027 0.021 – 0.033	0.021 0.019 – 0.024	0.024 0.023 – 0.026
Alkaline phosphatase, U/L	4.00 3.92 – 4.08	7.60 7.00 – 8.25	5.96 * 5.49 – 6.47

Notes: * – $p < 0.05$; *** – $p < 0.001$ compared to before treatment by Wilcoxon

sialic acids in the blood serum of this group of patients did not reach the level of clinically healthy persons. The redistribution of the fractional glycosaminoglycans composition in the group of patients who did not use laser therapy was as follows: the 1st fraction increased by 11.5%, the 2nd fraction decreased by 10.3%, the 3rd fraction did not change compared with the indicators before treatment (Table II).

Having analyzed the results of biochemical markers we observed a significant decrease in blood levels of glycoproteins, sialic acids and chondroitin sulfates in the group of patients treated with low-intensity infrared laser radiation and drug medications. After therapy, the content of glycoproteins decreased in blood serum by 28.9% and sialic acids lessened by 28.8%, which can be explained by a significant decrease in the activity of the joints inflammatory process during knee osteoarthritis. It should be noted that these rates did not differ from the values of clinically healthy subjects after the course of treatment, which indicates a higher efficiency of the treat-

ment regimen with the use of laser therapy than without it. It is obvious that the activity of the inflammatory process in the connective tissue of the joints under the influence of infrared laser radiation, diclofenac and glucosamine hydrochloride decreases faster and more significantly than in patients treated without laser therapy. Decrease in the activity of degenerative processes in cartilage tissue of joints of patients in the early stages of knee osteoarthritis was confirmed by the decrease in serum chondroitin sulfate serum concentrations by 91.3% compared with the indicator before treatment, mainly due to chondroitin-6-sulfate. The alkaline phosphatase activity was decreased by 36.1 % after therapy. This fact confirms the decrease in the activity of osteoblasts of the connective tissue of the affected joints. This suggests that the activity of osteoblasts of bone tissue begins to increase at the very beginning of the disease development. This means that without an appropriate early treatment using a complex of medical and physiotherapeutic measures, the pathological process in the

Table III. Dynamics of biochemical markers of connective tissue in patients with knee osteoarthritis during treatment with the use of laser therapy (Me, 25%–75%)

Biochemical markers	Control group, n=30	During the treatment, n=40	
		Before treatment	After treatment
Glycoproteins, g/l	0.60 0.48 – 0.72	0.90 0.87 – 0.92	0.64 *** 0.62 – 0.66
Sialic acids, mmol/l	1.92 1.61 – 2.23	2.92 2.08 – 3.05	2.08 *** 1.99 – 2.17
Chondroitin sulfates, g/l	0.078 0.072 – 0.084	0.243 0.227 – 0.270	0.127 *** 0.118 – 0.140
Total GAG, g/l	0.123 0.105 – 0.141	0.131 0.124 – 0.135	0.124 0.118 – 0.127
I fraction GAG, g/l	0.060 0.054 – 0.066	0.082 0.078 – 0.084	0.060 *** 0.057 – 0.062
II fraction GAG, g/l	0.036 0.028 – 0.044	0.030 0.028 – 0.031	0.036 0.035 – 0.037
III fraction GAG, g/l	0.027 0.021 – 0.033	0.019 0.018 – 0.020	0.026 * 0.020 – 0.028
Alkaline phosphatase, U	4.00 3.92 – 4.08	7.85 6.90 – 8.45	5.02 *** 4.42 – 5.41

Notes: * – $p < 0.05$; *** – $p < 0.001$ compared to before treatment by Wilcoxon

joints can lead to severe metabolic disorders from the side of the cartilage, and from the bone tissue. Thus, in assessing the degree of metabolic disorders in the body of patients at early stages of knee osteoarthritis, the decline in the activity of the enzyme alkaline phosphatase has not only a diagnostic but also an important prognostic value (Table III).

Thus, having compared two groups of patients with knee osteoarthritis treated with different regimens, it can be noted that the treatment scheme with low-intensity infrared laser radiation, sodium diclofenac and glucosamine hydrochloride was more effective compared to drug therapy. Therefore, the effectiveness of the complex treatment regimen using laser radiation and medication was confirmed by a more significant decrease in the biochemical markers of inflammatory-distractive changes in the connective tissue of the affected joints in patients with knee osteoarthritis.

CONCLUSION

The obtained results showed an increase in the content of glycoproteins by 55%, sialic acids growth by 54.7%, chondroitin sulfates increase by 3.3 times due to chondroitin-6-sulfate, and alkaline phosphatase growth by 94.5% compared with those in clinically healthy individuals, indicating the inflammatory and destructive changes in knee joints in the early stages of the disease, and requiring the appointment of integrated therapy. The patients treated only with diclofenac sodium and glucosamine hydrochloride showed the content of glycoproteins in blood serum decreased by 17.4%, sialic acids lessened by 18.6%, chondroitin sulfates decreased by 25.3%, and alkaline phosphatase activity slowed down by 21.6% after treatment compared with the indicators before treatment. The group of patients with knee osteoarthritis

treated with the use of laser therapy, diclofenac sodium and glucosamine hydrochloride showed the decrease in the content of glycoproteins by 28.9%, sialic acids – by 28.8%, chondroitin sulfates – by 91.3% due to chondroitin-6-sulfate, and alkaline phosphatase activity slowed down by 36.1% after treatment in comparison with the indicators before treatment. The above mentioned results showed a more significant decrease in the inflammation and destruction of the cartilage tissue of the affected joints in the treatment of knee osteoarthritis after application of laser therapy than without it.

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CORRESPONDING AUTHOR

Dmytro V. Morozenko

Sytenko Institute of Spine and Joint Pathology

80 Pushkinskaya Street, Kharkov, Ukraine

tel: +380677225748

e-mail: d.moroz.vet@gmail.com

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