SURGERY

DOI 10.35630/2023/13/3.311

Received 12 May 2023;
Accepted 15 June 2023,
Published 19 June 2023

BIOCHEMICAL SIGNS OF THE COURSE OF REPARATIVE OSTEOGENESIS IN PATIENTS WITH FRACTURES OF THE LOWER EXTREMITY

Andryi Kalashnikov , ***Yurii Litun*** ✉ ,
Yuriy Stavinskyi, Volodymyr Protsenko ,
Yevgen Solonitsyn

The Institute of Traumatology and Orthopedics NAMS of Ukraine, Kyiv, Ukraine

✉ ***litun_yurii@ukr.net***

download article (pdf)

UDC 616.71-001.58-07-08:001.891.5

ABSTRACT

Background: Some biochemical indicators in patients with disorders of reparative osteogenesis were described in a sufficient number of studies, but the role of factors affecting the metabolism of bone tissue and the ways of their correction are not sufficiently studied .. For effective prediction of the course of reparative osteogenesis in fractures, it is important to identify early markers of impaired consolidation and, accordingly, its pathogenetic correction.

Purpose: to determine biochemical indicators of the course of reparative osteogenesis in patients with multiple and monolocal fractures of the bones of the lower limb.

Materials and Methods: 44 patients aged 23 to 47 years were examined. To assess the ratio and intensity of the processes of biosynthesis and breakdown of collagen, as the major structural protein of connective tissue, the following indicators were determined in the blood serum of patients: fractions of hydroxyproline, oxyproline, collagenase, cathepsin B, elastase, proteolysis-antielastase inhibitors. In addition, indicators of mineral metabolism were also determined in the blood serum of patients: the content of calcium, phosphorus, alkaline phosphatase activity. **Results:** A comparative analysis and comparison of the results of the biochemical examination of patients with closed tibial fractures and multiple fractures revealed that already at the beginning of the research, deviations from the norm in the indicators of both mineral and collagen metabolism were observed. Both groups of the patients are characterized by the presence of disturbances in the ratio between calcium and phosphorus, and in patients with multiple fractures - an increase in the level of alkaline phosphatase. The detected changes in the mineral metabolism in patients with multiple fractures had a more pronounced and intense character due to the multiple injuries and presence of a large number of destroyed cells in the first days after the injury. ***Conclusions***: Indicators of the unfavorable course of reparative osteogenesis in patients with bone fractures are a decrease in the content of calcium/phosphorus in blood serum, an increase in the level of free oxyproline and collagenolytic enzymes (collagenase, elastase, cathepsin B). Besides, there is a decrease in the level of proteolysis inhibitors (antielastase, α_2-macroglobulin) and a decrease in suspension stability and antioxidant activity, increase in blood fibrinolytic activity. Our findings justify the need for prophylactic drugs that improve reparative osteogenesis soon after the injury (within a month) in patients with multiple fractures in order to activate the processes of reparative regeneration.

Keywords: reparative osteogenesis, fractures, biochemistry.

INTRODUCTION

According to the vast majority of authors, clinical and radiological signs of bone fracture healing in patients with multiple injuries of the musculoskeletal system differ from similar parameters in patients with isolated injuries [1, 2, 3]. As a rule, it takes longer for fusion of fragments in multiple bone fractures compared to monolocal fractures by 1.5-2 times [4, 5].

There are many methods of obtaining information about the bone tissue status: histomorphometric, densitometric, biochemical [6, 7] ones. Currently, biochemical research methods are used more often, as they are non-invasive, and they allow directly assessing the bone tissue status [8, 9, 10].

Some biochemical indicators in disorders of reparative osteogenesis have been described in a number of studies, but the role of factors affecting the metabolism of bone tissue and the ways of their correction are not sufficiently disclosed in them. For effective prediction of the course of reparative osteogenesis in fractures, it is important to identify early markers of consolidation disorders, as well as to create a comprehensive approach to the detection of such disorders and, accordingly, its pathogenetic correction.

The purpose of the study: to determine the biochemical indicators of the course of reparative osteogenesis in multiple and monolocal fractures of lower extremity bones.

MATERIALS AND METHODS

Biochemical studies were carried out in the laboratory of biochemistry of the Institute of Traumatology and Orthopedics NAMS of Ukraine (Kyiv, Ukraine). The study was conducted in accordance with the terms of the Declaration of Helsinki with the approval of the Ethic Commission of the Institute (Protocol No. 4 dated August 23, 2015). All patients participating in the study signed a voluntary informed consent statement.

44 patients aged 23 to 47 years were examined. The first group consisted of 20 patients (11 men, 9 women) who had closed fractures of lower-leg bones, the second group consisted of 13 patients (8 men, 5 women) with several (2-3) fracture locations, one of which was within the lower-leg bones. Among them, fractures of lower-leg bones were combined with thigh-bone fractures in 7 patients, 4 patients had bilateral bone fractures at the level of the lower-leg, and 2 persons, who underwent medical checkup, had fractures of lower-leg bones and of humerus. A biochemical study was performed on the second day after the injury (baseline), as well as in 1, 2, 3 and 4 weeks after it. The third group included 11 patients (4 men, 7 women) with delayed consolidation of tibial fragments after multiple fractures. The average period after injury in these patients was (6.3±0.4) months. The average age of patients in the first group was (31.2±3) years, in the second group - (29.3±3), and in the third group - (34.3±2) years.

In order to assess the ratio and intensity of the processes of biosynthesis and breakdown of collagen, as the major structural protein of connective tissue, the following indicators were determined in the blood serum of patients:

- hydroxyproline fractions (protein-peptide-bound and free oxyproline) were isolated using the Frey procedure, the oxyproline content at them was isolated using the modified Stegmann method. The amount of oxyproline was expressed in μmol of the amino acid in 1 liter of blood serum - μmol/l;
- collagenolytic enzymes (collagenase, cathepsin B, elastase) were expressed in μmol of cleaved oxyproline per 1 hour in a liter of blood serum - μmol/l*g. Inhibitors of proteolysis: antielastase was expressed in mg/l per second - mg/l*s; α1-proteinase inhibitor (α1-PI), and α2-macroglobulin (α2-MG) were expressed in g/l.

In addition, indicators of mineral metabolism were also determined in the blood serum of patients: the content of calcium, phosphorus, alkaline phosphatase activity.

Statistical analysis. Statistical data processing was carried out using the Statistica 12 package (StatSoft, USA). Descriptive statistics methods were used to display the general characteristics of the initial parameters, indicating the mean value and standard deviation. For variables with a normal distribution, group comparisons were performed using the Student's test. In order to determine the statistical significance of differences between groups, the Mann-Whitney test was used for quantitative (non-normally distributed) and ordinal variables, and the $\chi 2$ test and Fisher's exact test were used for qualitative ones.

RESULTS

The determination of early markers of impaired consolidation in patients with fractures consisted in the study of differences in mineral metabolism, collagen metabolism, the state of enzymatic systems responsible for its synthesis and breakdown in patients with multiple and monolocal fractures of the bones of the lower limb.

The baseline of biomechanical indicators was determined, which was studied on the second or third day after the injury.

7 days after the injury a decrease in the content of calcium compared to the norm was found in the blood serum of patients of the first group with closed fractures of the lower-leg bones. After which its level was equal to the norm until the end of the observations (Table 1).

The level of inorganic phosphorus at the beginning of the examination slightly exceeded the normal values. A week after the injury, the phosphorus content was approaching the upper limit of normal fluctuations, and after 4 weeks its concentration was equal to the norm. Alkaline phosphatase activity at all stages of observation was within normal parameters.

Dynamic changes in collagen metabolites in the blood serum of patients of the first group were quite clearly revealed. In the initial state, the level of protein-bound oxyproline was significantly (by 74.4%) higher than normal, during 3 weeks it gradually decreased, and at the end of the 4th week, the content of the fraction remained higher than normal by 18.7%.

The concentration of peptide-bound oxyproline, increased by 1.7 times at the beginning of the examination, remained until the end of the first week after the injury, after 14 days the content of the fraction gradually decreased and approached the upper limit of normal values after 4 weeks.

The initial level of free oxyproline was 2.2 times higher than normal. After 2 weeks, there was a gradual decrease in the concentration of free oxyproline in blood serum. After 4 weeks, the level of free oxyproline exceeded the norm by 67.2%.

When studying the dynamics of collagenolysis, significant and unidirectional changes in collagenolytic enzymes were found. In the first days after the injury, collagenase, cathepsin B, and elastase had a significantly higher level of activity compared to normal values (55.4%; 66.7%; 59.4%, respectively).

Table 1. Indicators of mineral and protein metabolism in the blood serum of patients with monolocal fractures of the lower limb bones at different periods of observation (M+m) and the reliability of the differences (p) in comparison with the baseline

Indicators	Norm	Baseline	Observation periods, weeks			
			1	2	3	4
Mineral metabolism						
		n=15	n=11	n=8	n=7	n=5
Calcium, mmol/l	2.12-2.62	1.95±0.12	2.08±0.10	2.32±0.24	2.44±0.24	2.23±0.06 p<0.05
Phosphorus, mmol/l	0.65-1.29	1.42±0.09	1.32±0.05	1.36±0.13	1.43±0.10	1.06±0.09 p<0.05
Alkaline Phosphatase, mmol/l*g	0.50-1.30	1.29±0.13	1.01±0.13	1.13±0.14	1.30±0.14	1.12±0.08
Protein metabolism						
Protein-bound oxyproline, μmol/l	41.90±0.76	73.21±5.49	60.86±4.88	52.08±4.35 p<0.05	48.48±2.32 p<0.01	49.72±1.83 p<0.01
Peptide-bound oxyproline, μmol/l	10.76±0.23	18.38±1.22	20.90±0.99	14.57±0.92 p<0.05	13.12±0.61 p<0.01	11.97±0.31 p<0.01
Free oxyproline, μmol/l	8.39±0.23	19.83±1.22	20.90±1.98	15.63±0.99 p<0.05	14.95±1.45 p<0.05	14.03±0.99 p<0.01

Collagenase, μmol/l*g	0.68±0.01	1.05±0.07	1.11±0.05	0.89±0.05	0.80±0.04 p<0.01	0.73±0.01 p<0.01
Cathepsin V μmol/l*g	0.84±0.02	1.40±0.06	0.99±0.05	1.07±0.04 p<0.01	0.88±0.04	0.92±0.03
		n=10	n=10	n=8	n=8	n=5
Elastase, mg/l*s	1.80±0.08	2.82±0.03	2.62±0.02 p<0.05	2.32±0.02 p<0.01	2.21±0.02 p<0.01	1.78±0.02 p<0.01
Antielastase, mg/l*s	3.40±0.22	1.86±0.02	2.16±0.03 p<0.05	2.60±0.02 p<0.01	2.70±0.02 p<0.01	3.14±0.01 p<0.01
α$_1$ – PI, g/l	1.95±0.01	2.51±0.01	2.64±0.03	2.29±0.01 p<0.01	2.23±0.02 p<0.01	1.90±0.02 p<0.01
α2-MG, g/l	3.30±0.12	1.46±0.03	1.69±0.03	2.73±0.03 p<0.05	3.01±0.03 p<0.01	3.20±0.02 p<0.01

7 days after injury, collagenase activity tended to increase relative to the baseline. After 2 weeks, the activity of the enzymes gradually decreased - up to the lower limit of the norm 4 weeks after the operation (respectively, 0.73 + 0.01 μmol/l*g and 0.65-0.72 μmol/l*g). The level of activity of cathepsin B one week after the injury was 1.3 times lower than the initial level, after 2 weeks it tended to increase, and after 3 weeks the activity of the enzyme reached normal values until the end of the observation.

Elastase activity at the beginning of the research significantly exceeded the normal level (by 1.6 times). But already in a week after the injury, it gradually decreased and at the end of the observation reached normal values.

Dynamic changes of proteolysis inhibitors were multidirectional in nature. In the first days after the injury, the level of α1- proteinase inhibitor (α1-PI) exceeded the norm by 1.3 times, after a week - an increase of 1.4 times was observed, then the concentration of this indicator decreased and reached the norm at the end of the observations. The content of α2- macroglobulin (α2-MG) at the beginning of the examination was 2.3 times lower than normal values. After 7 days, the level of the inhibitor gradually increased and after 4 weeks was equal to the norm.

Antielastase activity in the first days after the injury was 1.9 times lower than normal. After a week, the activity of the inhibitor gradually increased. At the end of the observations, the antielastase content in the blood serum was normalized.

The results of the biochemical examination in the patients with *multiple* fractures are presented in Table 2. The calcium content at the beginning of the studies was lower than normal, gradually increased and reached normal values 3 weeks after the beginning of the examinations. The content of inorganic phosphorus in the first days after the injury exceeded the norm, it remained high for 3 weeks, and only after 4 weeks its normal concentration in blood serum was noted in the patients. At the same time, the calcium/phosphorus ratio in blood serum was unfavorable for reparative regeneration after bone fractures. Alkaline phosphatase activity was elevated during 3 weeks, gradually decreasing and reaching normal values 4 weeks after the start of observations.

The study of the dynamics of collagen metabolites in the blood serum of patients showed that the level of protein-bound oxyproline increased (by 76.5%) in the first days after the injury. After a week, the content of the fraction decreased sharply to the limit of normal fluctuations, and was later slightly higher than normal.

The level of peptide-bound oxyproline at the beginning of the examination exceeded the norm by 1.8 times, remaining high enough during the 4 weeks of observation.

The content of free oxyproline, found in the first days after the injury, was significantly increased compared to the norm (by 2.6 times), remained at a high level and at the end of the observations was 1.8 times higher than the norm.

Table 2. Indicators of mineral and protein metabolism in the blood serum of patients with multiple fractures at different periods of observation (M+m) and the reliability of the differences (p) compared to the baseline

Indicators	Norm	Baseline	Observation periods, weeks			
			1	2	3	4
Mineral metabolism						
		n=12	n=11	n=8	n=5	n=6
Calcium, mmol/l	2.12-2.62	1.95±0.06	2.00±0.06	2.07±0.18	2.53±0.25 p<0.05	2.62±0.06 p<0.01
Phosphorus, mmol/l	0.65-1.29	1.52±0.04	1.53±0.04	1.37±0.13	1.53±0.06	1.20±0.08 p<0.01
Alkaline Phosphatase, mmol/l*g	0.50-1.30	1.52±0.10	1.44±0.08	1.47±0.11	1.44±0.13	1.09±0.12 p<0.05
Protein metabolism						
Protein-bound oxyproline, µmol/l	41.90±0.76	71.91±3.81	39.05±1.91	62.38±4.96	55.36±3.19 p<0.01	53.00±5.80 p<0.01
Peptide-bound oxyproline, µmol/l	10.76±0.23	19.14±0.99	22.34±1.32	19.52±1.07	19.83±1.14	18.07±1.22
Free oxyproline, µmol/l	8.39±0.23	22.04±0.24	21.58±1.37	20.14±1.45	18.45±2.14	15.02±1.53 p<0.01
Collagenase, µmol/l*g	0.68±0.01	1.17±0.07	1.12±0.06	1.26±0.01	0.09±0.08 p<0.05	0.80±0.02 p<0.01
Cathepsin V µmol/l*g	0.84±0.02	1.30±0.07	1.14±0.06	1.24±0.12	1.06±0.06 p<0.01	1.00±0.07 p<0.01
		n=9	n=9	n=8	n=7	n=5
Elastase, mg/l*s	1.80±0.08	3.22±0.04	2.78±0.03 p<0.01	2.93±0.02 p<0.01	2.44±0.03 p<0.01	2.27±0.01 p<0.01
Antielastase, mg/l*s	3.40±0.22	1.65±0.03	1.89±0.02 p<0.05	1.72±0.02	2.55±0.03 p<0.01	2.75±0.01 p<0.01
α_1 – PI, g/l	1.95±0.01	2.64±0.02	2.77±0.02	2.52±0.02	2.31±0.02 p<0.05	2.25±0.01 p<0.05
2-MG, g/l	3.30±0.12	1.19±0.03	1.89±0.01 p<0.01	1.71±0.02 p<0.01	2.42±0.03 p<0.01	2.70±0.02 p<0.01

The study of the dynamics of collagenolysis indicators revealed a unidirectional, but different in intensity, nature of shifts in collagenolytic enzymes during the entire period of observation (Table 2).

The initial level of collagenase and cathepsin B activity was significantly higher than normal values (72.1% and 54.8%, respectively), and remained high until the end of the 2nd week. After 3 weeks, there was a decrease in the catalytic activity of enzymes, but until the end of the observations, the activity of both collagenase and cathepsin B remained higher than normal (by 17.6% and 19.0%, respectively).

Elastase activity in the first days after the injury was 1.8 times higher than normal. After a week, a gradual decrease in the elastolytic activity of the enzyme was observed, but by the end of the observations, it was 26.1% higher than normal.

Dynamic shifts of proteolysis inhibitors in the blood serum of victims with multiple fractures were different in direction and intensity. The initial level of anti-elastase was 2.1 times lower than normal. A week after the injury, the inhibitory effect of anti-elastase gradually increased, but after 4 weeks it remained 1.3 times lower than normal values.

The content of α_1 – PI in the first days after the injury exceeded the norm by 35.4%, then it gradually normalized, almost reaching the upper limit of normal fluctuations by the end of the 4th week.

At the beginning of the examination, the level of the inhibitory capacity of α2-MG was 2.8 times lower than normal. After 7 days, the activity of the inhibitor gradually increased, but at the end of the observations it remained 18.2% lower than normal values.

In patients with delayed consolidation of tibial bone fragments after multiple fractures, the dynamics of changes in biochemical parameters was as follows (Table 3).

Table 3. Indicators of mineral and protein metabolism in the blood serum of patients with delayed tibial consolidation after multiple fractures before and after conservative treatment (M+m) and the reliability of the differences (p) compared to the baseline

Indicators	Norm	Before treatment	In 4 months after starting treatment
Mineral metabolism			
		n=7	n=6
Calcium, mmol/l	2.12-2.62	1.84±0.10	2.34±0.07 p<0.05
Phosphorus, mmol/l	0.65-1.29	1.05±0.11	1.11±0.14
Alkaline Phosphatase, mmol/l*g	0.50-1.30	1.15±0.13	1.29±0.13
Protein metabolism			
Protein-bound oxyproline, μmol/l	41.90±0.76	38.83±5.49	44.15±3.58 p<0.01
Peptide-bound oxyproline, μmol/l	10.76±0.23	12.96±1.60	11.38±0.84
Free oxyproline, μmol/l	8.39±0.23	14.96±1.76	11.55±1.39 p<0.001
Collagenase, μmol/l*g	0.68±0.01	0.85±0.04	0.64±0.10 p<0.05
Cathepsin V μmol/l*g	0.84±0.02	0.94±0.09	0.74±0.10 p<0.05
Elastase, mg/l*s	1.80±0.08	2.13±0.14	1.76±0.11 p<0.01
Antielastase, mg/l*s	3.40±0.22	2.74±0.12	3.16±0.07 p<0.05
α_1 – PI, g/l	1.95±0.01	1.71±0.15	1.90±0.12
α2-MG, g/l	3.30±0.12	1.74±0.22	3.26±0.33 p<0.001

At the beginning of the studies, serum calcium was significantly lower than normal, also a decrease in the calcium/phosphorus ratio, and a normal level of alkaline phosphatase were revealed. The study of collagen metabolism indicators revealed a slightly reduced content of protein-bound oxyproline and a slight increase in peptide-bound oxyproline compared to the norm. In contrast, the level of free oxyproline was 78% higher than normal values.

The activity of collagenolytic enzymes was higher than normal: collagenase – by 25%, elastase – by 18%, cathepsin B – by 12%. The content of proteolysis inhibitors in blood serum was reduced: anti-elastase - by 19%, α_1 – PI by 12% and α2-MG by 47%. After treatment, the level of calcium and protein-bound oxyproline significantly increased in the patients, the content of free hydroxyproline, collagenase, cathepsin B, elastase decreased, and the level of antielastase and α2-MG increased.

As a result of the treatment, all patients of the first group underwent fracture healing within 3.7±0.8 months. In 10 patients of the second group, the tibial bone consolidation period was 7.12±1.4 months. In 2 patients of the second group, fusion of the tibial bone did not occur within the specified time, which is why metal-osteosynthesis with bone autoplasty was subsequently performed on them. In all patients of the third

group, as a result of treatment, consolidation was established in 4.6±1.1 months.

INTERPRETATION

A comparative analysis and comparison of the results of the biochemical examination in patients with single and multiple fractures revealed that already at the beginning of the study, deviations from the norm of both mineral metabolism and collagen metabolism were observed. Both groups of patients are characterized by the presence of disturbances in the ratio between calcium and phosphorus, and in patients with multiple fractures, an increase in the level of alkaline phosphatase was noticed. The detected changes in the mineral metabolism of patients with multiple fractures had a more pronounced and intense nature and were possibly caused by the multiple injuries and the presence in the first days after the injury of a large number of destroyed cells [3, 5].

Normalization of indicators of mineral metabolism in patients with bone fractures of the lower limb occurred already in 1 week after the injury, while in patients with multiple injuries it occurred within 3-4 weeks after the injury. The study of characteristics of the collagen exchange status in the first days after the injury revealed a whole complex of interrelated changes, which can be considered as the response of connective tissue to traumatic bone damage.

At the beginning of the examination, both in patients with monolocal and in patients with multiple bone fractures in the settings of active collagen biosynthesis (which was evidenced by a significantly increased content of protein-bound oxyproline), a predominance of catabolic processes was observed (a sufficiently high level of free and peptide-bound oxyproline).

The mechanism of shifting the balance in the exchange of collagen towards the advantage of protein breakdown is due to disruptions of the enzyme-inhibitor balance in the proteolysis system. One of the main reasons for increased collagenolysis should be considered an increase in the activity of collagenolytic enzymes – of collagenase, cathepsin B and elastase. It is known that during reparative processes, cathepsin B exerts an activating effect on latent collagenase. The increase in collagenase activity is associated with the acceleration of the process of activation of procollagenase into collagenase due to increased activity of cathepsin B. Another, no less important factor is the reduction of the inhibitory capacity of α2-MG and anti-elastase [11, 12].

A high level of α_1 – PI against the background of a significant decrease in the inhibitory effect of anti-elastase and α_2-macroglobulin can be considered as a compensatory reaction in response to the hyperactivation of collagenolytic enzymes.

The study of collagen metabolism indicators in patients with monolocal fractures revealed a pronounced reaction to the presence of bone trauma and displacement of the organic base in the first days after the injury. The dynamics of the content of indicators in the organic basis of bone tissue (collagen metabolites, the activity of collagenolytic enzymes and their inhibitors) allows us to determine that the dynamics of bone callus formation due to the organic component of bone is quite intense. In patients with multiple bone fractures, the reparative process was more prolonged. It should be emphasized that the body's reparative response to the presence of various types of traumatic damage is uniform, according to the changes in the indicators that were studied. The mechanism of it is aimed at creating a bone callus through the formation of an organic base and its mineralization. Indicators of mineral and protein (collagen) metabolism revealed the same direction of regulation of the synthesis and breakdown of collagen in different groups of patients. The difference was only in the intensity of these changes, which depended on the severity of the traumatic injury, the time elapsed since the injury [4, 7].

The dynamics of changes in indicators of mineral metabolism, collagen metabolism, and the state of proteolytic activity, which was revealed as a result of the research, allows us to state that with monolocal fractures, the complete normalization of bone tissue metabolism after injury occurs after 3-4 weeks. Thus, in monolocal fractures, adaptive and compensatory resources of the body are quite sufficient to provide the necessary plastic substances for the process of reparative osteogenesis [13, 14].

In multiple fractures, normalization of mineral metabolism lasted much longer and was not completed till the end of the observations (a month after the injury). The level of free hydroxyproline, collagenase remains elevated. The imbalance in the ratio of calcium/phosphorus in blood serum remains. This can lead to an unfavorable course of reparative osteogenesis in the future. 6 months after injury, unidirectional metabolic shifts (consolidation disorders) were reported in patients with lower extremity fractures.

CONCLUSIONS

1. Thus, possible prognostic signs of an unfavorable course of reparative processes at the site of damage are a decrease in the ratio of calcium to phosphorus in the blood serum due to a decrease in calcium content, an increase in the level of free oxyproline, an increase in the level of collagenolytic

enzymes (collagenase, elastase, cathepsin B), a decrease in level of proteolysis inhibitors (antielastase, α_2- macroglobulin).

2. Our findings indicate the need for prophylactic drugs that improve reparative osteogenesis in multiple fractures soon after the injury (within a mont*h)* in order to optimize the processes of reparative regeneration and prevent the disorders of reparative osteogenesis.

CONFLICT OF INTEREST AND FINANCIAL SUPPORT

The authors declare that there is no conflict of interest and financial support in obtaining the results and writing this article.

AUTHOR CONTRIBUTIONS

A.V. Kalashnikov – research concept and design, analysis of data collection; Y.M. Litun - collection and processing of material, analysis of research data, writing the text; Y.O. Stavinskyi - collection and analysis of data from literary sources, selection of patients, conducting statistical studies.

REFERENCES

1. Mills L.A., Aitken S.A., Simpson A.H. The risk of non-union per fracture: current myths and revised figures from a population of over 4 million adults. *Acta Orthop*. 2017;88(4): P. 434–439. DOI: 10.1080/17453674.2017.1321351
2. Diaphyseal long bone nonunions – types, aetiology, economics, and treatment recommendations / M. Rupp, C. Biehl, M. Budak et al. *International Orthopaedics (SICOT).* 2018. Vol. 14, № 42. P. 247–258. DOI: 10.1007/s00264-017-3734-5
3. Riabenko T.V. Modern aspects of reparative regeneration of bone tissue and analysis of bone metabolism indicators. *Ukrainian Journal of Medicine, Biology and Sports.* 2020. V. 5, No.3. p. 89–97. https://doi.org/10.26693/jmbs05.03.089
4. Zura R., Mehta S., Della Rocca G.J. Biological risk factors for nonunion of bone fracture. *JBJS Rev*. 2016. P. 516–525. DOI: 10.2106/JBJS.RVW.O.00008
5. Hnateiko N.O. The role of multidimensional markers of osteogenesis in the diagnosis of bone fracture consolidation disorders and the principles of their prevention: thesis. … Ph.D.: 14.01.21 / ITO NAMSY. Kyiv, 2016. p.173
6. Buza A., Einhorn T. Bone healing in 2016. *Clin Cases Miner Bone Metab*. 2016. No 13. P. 101–105. DOI: 10.11138/ccmbm/2016.13.2.101
7. Calori G., Mazza E., Mazzola S. Non-unions. *Clin. Cases Miner. Bone Metab*. 2017. No 14. P. 186–207. DOI: 10.11138/ccmbm/2017.14.1.186
8. Ghiasi M., Chen J., Vaziri A. Bone fracture healing in mechanobiological modeling: a review of principles and methods. *Bone Reports.* 2017. No 2. P. 13–27. DOI: 10.1016/j.bonr.2017.03.002
9. Buza J.A., 3rd, Einhorn T. Bone healing in 2016. *Clin Cases Miner Bone Metab*. 2016. No. 13. P. 101–105.
10. Emara K.M., Diab R.A., Emara A.K. Recent biological trends in management of tracture non-union. *World J Orthop*. 2015. No 6. P. 623. DOI: 10.5312/wjo.v6.i8.623
11. Garnero P. New biochemical markers of bone turnover / P. Garnero // IBMS Bonekey. – 2008. – Vol. 5, No 3. – P. 84-102.
12. Barkov A.V., Barkov A.A. Resumption of processes of reparative regeneration in patients with nonunion fractures of the femur. *Ukrainian morphological almanac*. 2010. No. 2. p. 11–13.
13. Povorozniuk V. V., Makohonchuk A. V. Post-traumatic osteopenia in fractures of long tubular bones. *Injury.* 2014. No. 2. p. 25–28.
14. Weber B., Cech O.˘ Pseudarthrosen: Pathophysiologie, Biomechanik, Therapie, Ergebnisse; Huber: Edison, NJ, USA, 1973. 89 p.

back