

HOMEOMESOTHERAPY OF THE PATHOLOGICAL SCARS

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Research aims. The aim is to detect indications and efficiency, when using the method of mesotherapeutic impact of preparations Made and Collagen-Guna on the pathological scars of different origin and age in comparison with traditional methods.

Materials and methods. Patients with pathological scars - keloid, hypertrophic and atrophic scars (KS, HS, AS), who was treated at Kharkiv burn center from November 2009 to July 2011, were taken under clinical observations. To the first (basic) group belonged patients with pathological scars of different age which embrace different areas, they only got mesotherapeutic treatment with antihomotoxic preparations Made and Collagen-Guna, the second group was a control one, similar patients received a standard treatment regimen. The efficiency of treatment was estimated in accordance with clinical characteristics of scar tissue, patients' subjective estimation, laboratory blood analysis and histological study of scar tissue before the treatment and at the end.

Findings and Discussion. *The first group* (30 patients aged 10 to 62) was only treated with intralesional injections course of preparations Made and Collagen-Guna in amount of 0.5 - 4 ml/week. None of the patients was treated with the compression therapy because of scars location, the conservative therapy of joints desmogenic contracture was not conducted and medium term of treatment was 115 days. After 2 weeks of treatment the softening and flattening of HS and KS, the minimization of vegetative reaction were noticed; lightening of scar tissue, disappearance of the scar tissues flabbiness in patient with AS and color changes from depigmentation to skin color were noticed after 4 weeks. After 2 weeks of treatment all the patients noticed pain and itch relief subjectively. At the end of the treatment, the scar tissue is soft, elastic, without any pathological vegetative reaction, it can be easily taken into a fold, in patients with HS and KS the color of skin is from flesh to intensive pink (in case of old KS), in patients with AS the skin has flesh color. The most evident transformation of scar tissue ensued in patients with fresh scars (the beginning of treatment not later than after 1 – 2 month from the start of scar tissue growth) in period of 80 days, in

patients with old scars (the first visit to a doctor in 8 – 12 months from the beginning of scar tissue growth) in period of 125 days. Complications and side effects were not found out during the treatment procedures painfulness is moderate. Clinically the scar tissue, which takes up a small area, becomes hardly noticeable, similar to the surrounding skin. The extensive scar tissues slightly differ in color from the not injured skin, but functionally they are identical to it, what shows itself by gain of motion in the joints with desmogenic contractures.

In the control group (15 patients aged 14 to 45) a standard anti-scars therapy was performed it was compression therapy (elastic pressure bandages), ointment Contractubex, physiotherapy courses, a course of Longidaza intramuscularly № 20, remedial gymnastics, intralesional injections of steroid hormones, sanatorium-and-spa treatment. The medium term of treatment makes up 218 days. Clinically, at end of treatment scar tissue is soft, with low-grade vegetative reaction, can be taken into a fold not on the all areas of the skin, color varies from hyperpigmented to intensive pink. Patients with AS have following changes: increased flabbiness of scar tissue, persistent depigmentation remains in scars.

According to the ultrasound scan results before the treatment the height of hypertrophic and keloid scars averaged 4.31 mm, the depth of atrophic scars made up 3.05 mm. The presence of not uniform irregular-shaped inclusions with reduced echogenicity in the scar tissue was diagnosed in all the patients; there is strong flattening or absence of a dermo-epidermal junction line. At the end of treatment the scar height (HS, KS) averaged 3.43 mm, the depth of AS averaged 4.2 mm; the order of fibers and the evident dermo-epidermal junction line are observed in the scars structure. Thereby the height reducing of hypertrophic and keloid scars averaged 0,872 mm ($d < 0,05$), and the depth increase of AS averaged 1,15 mm ($d < 0,05$).

There are similar results in the control group, treatment performed at the same time, they keep unimportant small difference in indexes in patients with HS and KS, but they differ significantly in patients with AS – Table 1.

Table 1.**The ultrasound scan results of pathological scars before and after the treatment.**

Research groups	Before the treatment, mm		After the treatment, mm		Changes, mm, %	
	HS, KS	AS	HS, KS	AS	HS, KS	AS
Basic group	4,31	3,03	3,43*	4,2***	- 0,872***, -20,42%	+1,15*** +38,61%
Control group	4,28	2,89	3,58*	2,32*	-0,70*, -16,36%	-0,57*, -19,73%

Note: * The differences are significant $D < 0,05$, ** – differences are significant between the groups.

Thus, when using the both treatment methods, the height reducing of HS and KS occurs with a significant small difference, and when treating AS in the basic group of treatment the volume filling of lost tissue occurs, what is physiologically positive, and when treating with standard methods the atrophy of the tissue get worse on the contrary.

Morphological examination of biopsy materials of the old scar tissue in the basic group before the treatment (100, 200 and 400, hematoxylin and eosin stain and Van Gieson's stain): papillary layer of dermis became thin, it is not full-blown, there is a tough fibrous connective tissue with occurrence of hyalinosis in the reticular dermis; almost complete absence of vascular component attracts attention – single large thick-walled vessels with sclerosis and hyalinosis of their wall. Reticular layer has a zonal structure with the presence of beams of tough fibrous sclerosed fibers that are random woven and complete lack of cellular elements (fibroblasts and fibrocytes), these bundles are surrounded by a dense enough and cellular connective tissue and with a relatively large number of fibroblasts and a small amount of thin-walled vessels. After the treatment the reticular dermis is presented with a fibrous, soft, well vascularized connective tissue with a small amount of coarse-fibered collagen. The

basis of well-defined, interlacing collagen fibers is a large quantity of fibroblasts and fibrocytes. Rather more of them can be observed around small, thin-walled vessels. In this case, the morphology of the dermis connective tissue approximates to the structure of the skin with normal structure.

Morphological research in the control group was carried out only at the end of treatment: hyperkeratosis laminated pavement epithelium, flattening of dermo-epidermal line, growth of coarse sclerosed fibers in the reticular dermis, poor leucocytic infiltration, isolated vessels and fibroblasts. Histopathologic finding of biopsy in the control group have expressed differences from that in the basic group.

The purpose of laboratory blood analysis before and after the treatment was to study the metabolism of connective tissue in the process of anti-scars therapy conducting and identification of potential diagnostic indicators of pathological process activity cicatrization. We studied the free and bound oxyproline, total collagen, glycosaminoglycans (GAGs), vitamin C, flavin adenine dinucleotide, L-tryptophan, core-protein in both groups of patients, who was receiving treatment of pathological scars. Comparison was carried out with information about laboratory norms of conventionally healthy people. One of the main indicators of collagen metabolism is the content of oxyproline in the blood. Oxyproline (proline derivative) is one of the collagen basic amino acids, this allows us to consider it as a marker, which reflects the catabolism of this protein. About 20% of peptides, which contain oxyproline released from the collagen molecules, are excreted with the urine. Only 1% of urine oxyproline is in free form, the remaining 99% are the peptides' components. In case of violation of collagen synthesis the amount of cross-links in collagen fibrils decreases, what leads to an increased content of easily soluble collagen. Therefore, in patients with impaired metabolism of connective tissue the excretion of oxyproline increases in urine, in the blood the content of its free fraction increases and the content of its bound fraction reduces.

GAGs play an important role in the transport and exchange of water, salts, nutrients and metabolites in tissues. The study of structural and metabolic state of the connective tissue discovered high activity of GAGs, which amount was largely

increased in the blood plasma of patients in the 2 and in the 1 group respectively ($57,5 \pm 2,4$ and $49,2 \pm 1,3$ micromole/l) after our treatment, 1,5 and 1,2 times higher respectively than, the indices of the comparison group (conventionally healthy patients). In the patients of both groups levels of GAGs increased before the treatment, although they were not for sure and statistically different, but they differed as compared with conventionally healthy group of observations.

Collagenolytic activity (CLA) had similar dynamics and didn't depend on the choice of therapy method. The highest CLA values were discovered in patients with the standard therapy method ($46,8 \pm 2,3$ micromole of oxyproline l/h). Levels of plasma's CLA in this group exceeded the data of conventionally healthy patients 7-8 times, and in the main group of 4-5. Thus this figure can be viewed as one, which has an important diagnostic and prognostic value in determining predisposition to a pathological cicatrization and, of course, for choice the method of therapy.

The average of free oxyproline in both groups before the treatment was $2,17 \pm 0,65$ micromole/l, while in 12 persons (41,5%), its concentration was increased, in 8 patients (35,6%) it was reduced and in the rest of patients oxyproline level was $1,55 \pm 0,27$ micromole/l, which fitted the indicators of conventionally healthy people. Also in the 1st and 2nd group there was a significant increase of bound oxyproline in serum compared to the indicators of control individuals (27,3% and 46,6%), respectively ($d < 0,001$). If we take into account that the level of free oxyproline in the blood serum reflects the intensity of the collagen decay, and the level of a protein-bound oxyproline reflects the activity of proliferative processes in the connective stroma of the organs (Г. Каминская., Н.Л. Пуряева, 1990, Н.Б. Содикова 2002), so the indices of the above mentioned markers in the pathogenesis of the pathological scars formation becomes clear.

Table 2

**Dynamic of biochemical parameters in patients with pathological scars
depending on the method of the treatment.**

Group and method of treatment	Indices, M±m							
	free oxyproline millimole/l		bind oxyproline mole/l		GAGs Micromole/l		CLA (micromole of oxyproline/1·h)	
	Befor e the treatm ent	After the treatmen t	Befor e the treatm ent	After the treatme nt	Befor e the treatm ent	After the treatmen t	Befor e the treatm ent	After the treatmen t
1 th group (basic) n = 27	1,32± 0,8	1,57±0, 3***	7,35± 1,4	7,7±1,3 ***	57,5± 2,4	52,7±4, 3*	31,7± 3,5	39,6±4, 2***
2 th group (control) n = 13	2,21± 0,5	2,1±0,2 *	9,5±1, 0	9,2±1,3 *	41,5± 1,9	49,2±1, 3***	46,8± 2,3	47,4±2, 5*
Conventio nally healthy people n = 18	1,49±0,12		6,28±0,19		38,2±1,2		Male – 7,3±0,56 Female – 7,6±0,43	

Note: * The differences are significant $D < 0,05$, ** – differences are significant between the groups.

The analysis of the research results of these parameters allows us to make following conclusions: **the formation of pathological scars is accompanied by profound metabolic disorders of connective tissue and is confirmed by a significant increase of collagenolytic activity of blood serum and glycosaminoglycans content in it.** Studies evaluation of oxyproline showed the most pronounced changes of bind oxyproline after the treatment, whereas the rates of free oxyproline were multidirectional. Almost half of the patients in two groups showed an increase of this index, which indicates an increased level of connective tissue remodeling. In other patients, against the background of basic therapy the free oxyproline levels were low, what, on the contrary, reflects the reduction in the intensity of collagen metabolism,

perhaps as a result of therapy we carried out. The GAGs index may be predictive criterion for the selection of therapeutic measures. The high correlation between **the dynamics of collagenolytic activity and the level of bind oxyproline of blood plasma allow us to use them as monitoring criteria for the effectiveness of the conducted therapy.**

Catabolism of connective tissue is carried out in the intercellular substance under the influence of specific enzymes - collagenase, elastase, proteases, glycosidases. These enzymes produce the same connective tissue cells, which are involved in the synthesis of these polymers.

Peptide chains of collagen are forming on polyribosomes, associated with the membranes of the endoplasmic reticulum (EPR). Simultaneously with the translation of DNA hydroxylation of proline and lysine residues takes place in peptide chains. Ascorbic acid acts as a reducing agent, which contributes to the hydroxylated iron in the ferrous state.

Hydroxylation of proline is necessary for the formation of a stable triple helix structure of collagen. Hydroxylation of lysine is necessary for the formation of covalent bonds between molecules of collagen in the formation of collagen structures. Hydroxylysine residues are sites of glycosylation. In case of vitamin C deficiency the collagen synthesis breaks down at the stage of hydroxylation. Less strong and less stable collagen fibers emerge.

As a result of our investigation, we established that patients who were in the basic treatment group had a significantly important increase in the level of vitamin C during therapy, than the 2th group, and in both groups without additional treatment (Fig. 1).

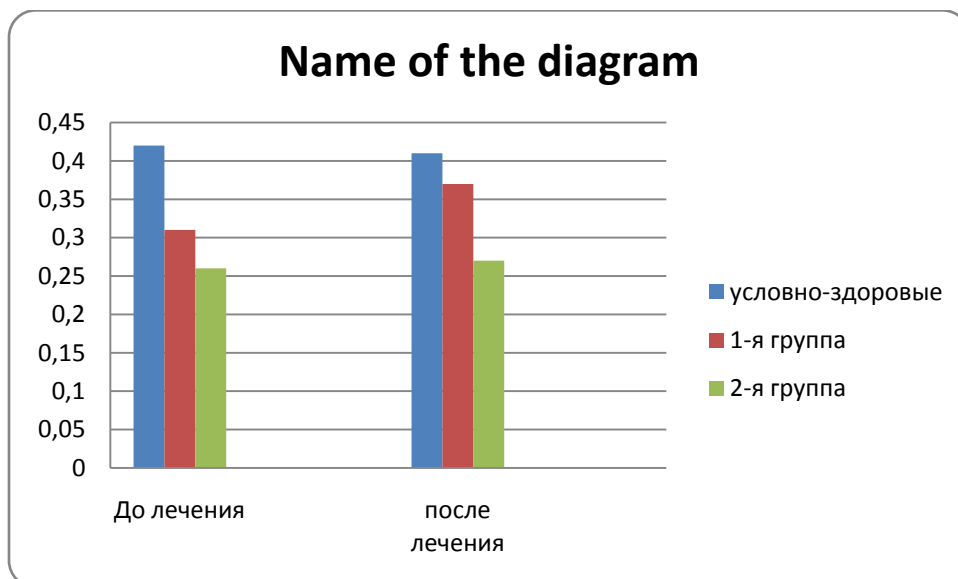


Fig. 1. Vitamin C Levels in patients with pathological scars depending on the treatment method.

Proteins-proteoglycans are called core-proteins. The protein part of proteoglycans, as well as of other secretory proteins is synthesized on the poliribosome EPR. Peptide chain penetrates the membrane and grows into the cavity of the EPR. Here begins the synthesis of glycosaminoglycans and proteoglycans part. As a result of our study of core-protein before the treatment, we found out that there is a qualitative positive reaction in the two studied groups (positive/positive), whereas in the group of conventionally healthy people, this reaction was negative (negative/negative) On the background of our therapy in the 1th group with basic treatment we saw reaction changes for (negative/positive) in the 2th group of patients reaction on the core-protein remained unchanged (positive/positive). Our data can indicate **the impact of our treatment on the first reactions of generation of the carbohydrate component of proteoglycans, which occurs in the EPR.** Most of the subsequent stages of synthesis glikozamin chains and their modifications occur in the Golgi apparatus, where it is much harder to influence biochemical processes. In the synthesis of GAGs participate corresponding nucleotide derivatives of monosaccharides and highly specific glycosyltransferase. For studying their effect we measured levels of FAD / FADH and copper content in the serum of patients before therapy and after it. As a result of our study we **found out a violation of metabolism oxidative stage of connective tissue at the level of FAD/FADH** as mandatory

participants of redox reactions and regulators of tissue respiration in the pentose-phosphate pathway, which runs at the moment of the pathological scars formation.

In the patients of both groups before the treatment, an oxidized form increase of coenzymes of flavin-dependent form. At a deeper study of bioenergetic processes in the cell, we analyzed the ratio of FAD and FADH, as regulators of the mitochondrial respiratory chain. In all the groups the reduction of FADH (0,0294±0.002 micromole/l) by the control values of (0,054±0,001 micromole/l) was observed. The concentration of FAD (0,234±0,13 micromole/l) in both groups was significantly higher ($d < 0,05$) than in the group of healthy patients (Table 3).

Table 3.

The concentration of FAD and FADH in the serum of patients with pathological scars (micromole/l) before the treatment

Indices	Standard	1 th group	2 th group
FAD+	0,243±0,13	0,365±0,08*	0,43±0,2*
FADH ₂	0,054±0,005	0,0294±0,001*	0,0267±0,004*
FAD+/ FADH ₂	0,09±0,02	0,12±0,035	0,15±0,028

Note: * the difference is reliable by comparison with the standard, ** - the difference is reliable between the groups

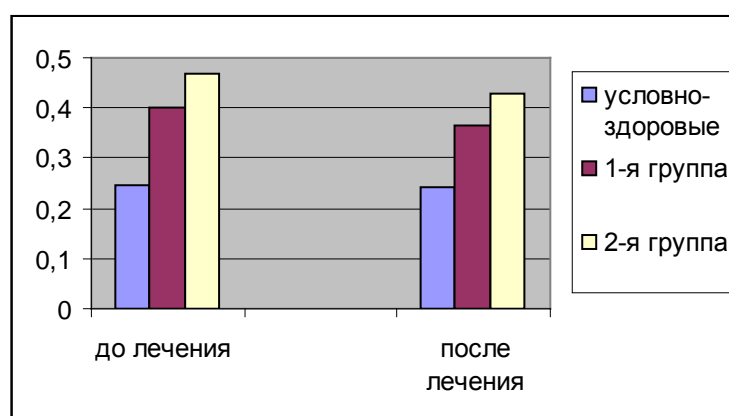


Fig. 2. The FADH levels in patients with pathological scarring before and after the treatment

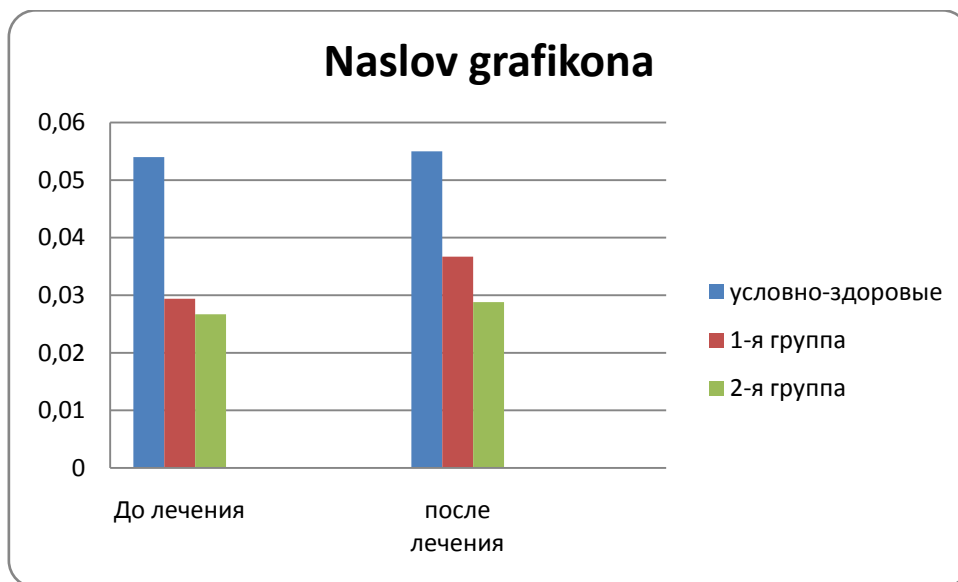


Fig. 3. The FADH₂ levels in patients with pathological scarring before and after the treatment

The correlation grows of FAD/FADH lowers the activity of FADH dependent enzymes in the cytosol and mitochondria. During the study of these data before and after our treatment, we found out that in the first group with the basic treatment the rates of FAD and FADH tended to improve, while in the second group they remained at the same level.

All this may indicate a reduction of dihydroxyacetone phosphate, an intermediate metabolite of glycolysis and gluconeogenesis, what leads to a rate reduction of gluconeogenesis and the starting of the pentose phosphate pathway, and consequently, increased formation of pathological scars in the second group of patients. The growth of FADH concentration, in comparison with FAD, slows down the oxidation in the pentose-phosphate pathway, thereby the ratio of lactate/pyruvate is increasing, what further reduces the rate of gluconeogenesis, and increases the lactate concentration in blood. Oxidative decarboxylation of pyruvate is accompanied by the formation of FADH and the Kynurenic pathway and its end products are directly dependened on the concentration of FAD, which is involved in the mitochondrial respiratory chain and provides the ATP cell. It is known that the ratio of FAD/FADH in the cell is a relatively stable rate and the reduction of FADH reduces the speed of decarboxylation of pyruvate. Thus, the rate of change in the ratio of FAD / FADH is an important factor, which represents the energy needed of cells

by regulating the rate of oxidation in the mitochondria and being responsible for the mechanism of the pentose phosphate pathway regulation.

Tryptophan is a source of nicotinamide coenzyme forms (NAD^+ and NADP) of vitamin B₅, as well as the tryptophan metabolism is associated with the formation of biogenic monoamine serotonin, hormone melatonin, inducer of cell differentiation and proliferation, - 5-hydroxyindoleacetic, 5-HIAA (5-hydroxy indolic acid), which are able influence considerable on the metabolism of various organs and tissues. Analysis of scientific data shows that the studies of the tryptophan metabolism and pathogenic role of its metabolic products in the mechanisms of pathological scarring development have not found the proper reflection in the available scientific literature. This issue is of great scientific interest to study the pathogenic mechanisms of pathological scars formation, diagnostic optimization of difficulty degree of the process of cicatrization and development of the appropriate treatment.

It is known that L-tryptophan is the stabilizer of the TDO (tryptophan-2 ,3-dioxygenase) enzyme. It contributes to formation of a stable conformational state, the TDO has an absolute substrate specificity towards the L-tryptophan and catalyzes the irreversible key reaction of amino acid catabolism in Kynurenic pathway of its metabolism with the formation of N-formyl-L-kynurenine, and later one of the key end-metabolites – NAD^+ . This enzyme accelerates the incorporation of molecular oxygen directly into the molecule of L-tryptophan and its catalyzed reaction is a factor which limited the speed of reaction of the conversion of the the substrate.

The examination has found out the activity growth of the TDO and the L-tryptophan content in the serum in the both groups before the treatment. In these metabolic conditions the way of increased synthesis of NAD^+ and NADP^+ coenzyme forms opens, needed for strengthening of the reducing syntheses of tissue differentiation and proliferation. The regulation of TDO is put into effect by the feedback of Kynurenic pathway final products of L-tryptophan NAD^+ and NADP^+ metabolism, whereas the enzyme activation is associated with an increase of the substrate oxidation content – L-tryptophan. Positive activators of the TDO enzyme are Cu^{2+} ions, hematin, ferriheme and σ -aminolevulinic acid (σ -ALA). Hemin is a TDO. A

significant increase in the TDO activity allows us to judge about the reducing of protein synthesis function of connective tissue in patients with pathological scarring and, and particularly about the disturbance of hemoglobin synthesis, what caused heme to oxidize by oxygen in hemin, which is a coenzyme activator of this enzyme, and on the other hand – the oxidized form of heme (hemin) inhibits the activity of the mitochondrial enzyme σ - aminolevulinic, which catalyzes the first reaction of heme synthesis from succinyl-CoA and glycine, σ - aminolevulinic acid.

The main suppliers of reduced substrates are the central metabolic pathways - oxidative decarboxylation of pyruvic acid and citric acid cycle. Both of them are realized in the mitochondria matrix, in the course of this processes occur reactions of decarboxylation (the most part of all the carbon dioxide, which is produced in the cells, is produced here). In addition, as already mentioned, during these processes the reactions of substrate dehydrogenation takes place, the reduced coenzyme forms $\text{NADP}\cdot\text{H}^+$ and FADH_2 are generated, their hydrogen comes into the respiratory chain of the internal mitochondria membrane, where its oxidation by oxygen to water and synthesis of ATP occurs. The growth of NAD^+/NADP , FAD/FADH_2 correlation indicates energy deficit and is a signal for the oxidation acceleration in the Krebs cycle. The main effect of the regulators is directed on the activity of three key enzymes: citrate synthase, isocitrate dehydrogenase and α -ketoglutarate dehydrogenase.

During the examination of L-tryptophan metabolism in both groups before the treatment, any statistically significant changes in the dynamics of L-tryptophan content in serum and the TDO enzyme activity ($D < 0.05$) were not found out, although the stable dynamics in increasing of L-tryptophan and TDO. However, against the background of the conducted therapy the parameters of L-tryptophan and the enzyme TDO activity differed significantly in the patients of the 1th group: they were $55,12 \pm 2,3$ micromole/l and $38,5 \pm 2,1$ nanomole kynurenine/mg of protein·1 hour for the L-tryptophan and enzyme TDO activity respectively, and in the 2th group $59,192 \pm 2,5$ micromole/l and $40,1 \pm 2,1$ nanomole kynurenine/ mg of protein·1 hour.

Analysis of the metabolic rates dynamics of amino acid L-tryptophan metabolism indicates that **the availability of L-tryptophan in serum is a significant diagnostic indicator of the possible pathological scarring**. These data **confirm clearly the important role of neuroendocrinal regulation in the pathogenesis of pathological scarring formation**. However, the evaluation of metabolites of L-tryptophan metabolism allows us to make a predictive conclusion about the level of activity or probability of pathological cicatrization.

Table 4

Indices of L-tryptophan metabolism in patients of the both groups with pathological scars before the treatment (M ± m)

Indices	group of observations, sex	Conditionally healthy people	
	Patients n = 32	Male (n = 23)	Female (n = 20)
L-tryptophan (micromole/l)	69,18±3,6*	51,8±2,3	50,5±3,0
TDO (nanomole kynurenine/mg of protein·1 hour)	41,6±4,1*	37,5±2,3	35,8±3,4

Note: * The differences are significant $D < 0,05$

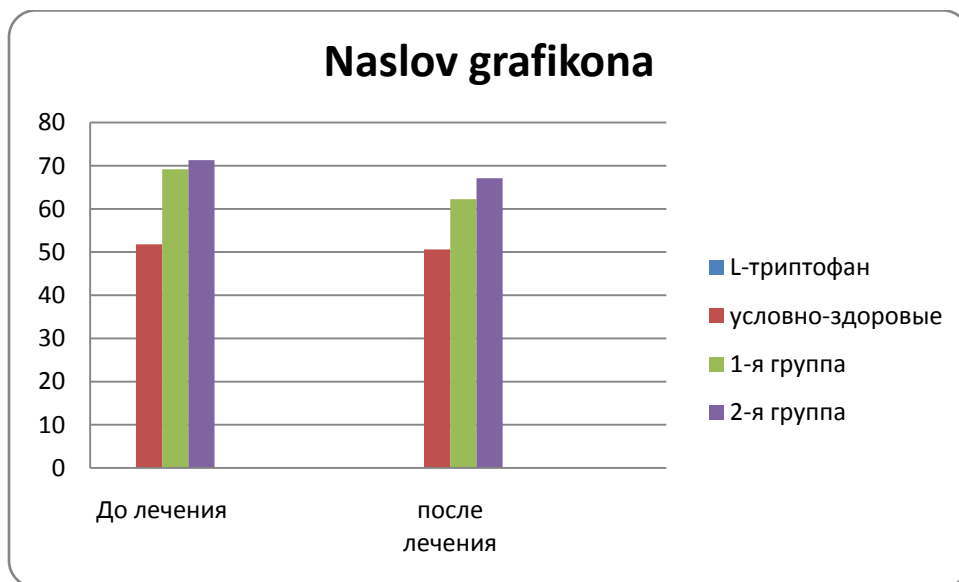


Fig. 4. L-tryptophan levels in patients with pathological scars before and after the treatment according to the treatment groups.

Thus, the study of amino acid L-tryptophan metabolism makes it possible to confirm objectively the stage of pathological scar development and monitor during the treatment process. The monitoring indicators are: the testing of L-tryptophan content in serum and the TDO enzyme activity, which reflects one of the important links in the structural and metabolic disorders during the development of pathological cicatrization.

The laboratory study of the indices of connective tissue metabolism makes it possible to optimize the pathogenetic therapy of pathological scars, namely: the inclusion of therapeutic and health promotion programs, which are aimed at normalization of the neuroendocrine regulation of L-tryptophan metabolism, organism detoxification, correction of metabolic acidosis, increasing the level of antioxidant protection and inhibition of oxidative stress, increasing of the immunological resistance in conjunction with local impact. The efficiency control of remedial measures can be realized by studying the metabolites dynamics of the amino acid L-tryptophan metabolism, what has a great prognostic value of the cicatrization process result.

Conclusions. The results of the conducted study demonstrate the high clinical efficiency of the presented mesotherapeutic treatment technology of pathological scars compared to the traditional treatment methods, such as:

- 1) Reducing treatment time at the average by 93-146 days.
- 2) Reducing the costs of reconvalescents' rehabilitation.
- 3) Achieving the best possible treatment outcome in the basic group with early start of treatment.
- 4) Getting clinically more full-fledged result when working with biologically safe drugs.
- 5) Determination of laboratory diagnostic indicators, which make it possible to control the treatment and make projections.
- 6) Identification of new trends in the treatment of patients with pathological scars.
- 7) Reduction of indication for the further conservative and surgical rehabilitation of reconvalescents.
- 8) Improving the patients' quality of life, optimization of social rehabilitation.

Mesotherapy of pathological scars with antihomotoxic preparations Made and Collagen-Guna can achieve significant functional and aesthetic results in a shorter period of treatment, it doesn't have any complications or side effects, any special requirements for equipment, the treatment course does not violate a way of life and work of patients, treatment results help to improve the quality of patients' life.