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HOMEOMESOTHERAPY OF THE PATHOLOGICAL SCARS

SUMMARY

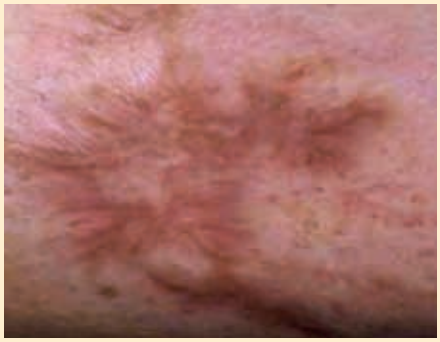
The results of this study concerning homeomesotherapy of the pathological scars demonstrate the high clinical efficacy compared to the traditional treatment, such as:

1) Reducing treatment time at the average by 93-146 days.
2) Reducing the costs of 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 medicines.
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.
8) Improving the patients' quality of life, and optimization of social rehabilitation.

Mesotherapy of pathological scars with Made® and Guna®-Collagen can achieve significant functional and aesthetic results in a shorter period of treatment; it doesn't have any complication or negative side effect, nor any special requirements for equipment is needed. The treatment course is not problematic fot the patients' life and work activities, and the treatment helps to improve their quality of life.

KEY WORDS

PATHOLOGICAL SCARS, HOMEOMESOTHERAPY, COLLAGEN, OXYPROLINE, GAGs, MADE®, GUNA® COLLAGEN



From: http://www.scars1.com/gallery/images/3wk_pre.jpg

RESEARCH AIMS

The aim of this research is to detect indications and efficiency, using the *low dose* preparations **Made®** and **Guna®-Collagen** (Guna Laboratories, Milan - Italy) in mesotherapy on the pathological scars of different origin and age in comparison with traditional methods.

MATERIALS AND METHODS

Patients with pathological scars – keloids, hypertrophic, and atrophic scars (**KS**, **HS**, **AS**) – who were treated at the Kharkiv Burn Center (Kharkiv-Ukraine) 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; these only got mesotherapeutic treatment with **Made®** and **Guna®-Collagen**.

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 and at the end of the treatment.

FINDINGS AND DISCUSSION

▶ The basic group (**30 patients** aged between 10 and 62 years) was treated only with an intralesional injections course of the preparations **Made®** and **Guna®-Collagen**, 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. The 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 and it can be easily taken into a fold.
In patients with HS and KS the color of the skin is from flesh to intensive pink (in case of old KS); in patients with AS the skin has a flesh color.
The most evident transformation of scar tissue ensued in patients with fresh scars (the beginning of treatment not later than 1-2 months from the start of scar tissue growth) in a period of 80 days, in patients with old scars (the first visit took place 8-12 months from the beginning

of the scar tissue growth) in a period of 125 days.
Complications and side effects were not found out; during the treatment procedures the painfulness was 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; which shows itself by gaining of motion in the joints with desmogenic contractures.

► In the control group (15 patients aged between 14 and 45 years) a standard anti-scars therapy was performed: compression therapy (elastic pressure bandages), ointment Contractubex, physiotherapy courses, a course of Longidaza N° 20 intramuscularly, gymnastics, intralesional injections of steroid hormones, sanatorium-and-spa treatment.

The medium term of treatment lasted 218 days.
Clinically, at end of the treatment, the scar tissue is soft, with low-grade vegetative reaction, cannot be taken into a fold on all the areas of the skin, color varies from hyperpigmented to intensive pink. Patients with AS present the following changes: increased flabbiness of scar tissue, and persistent depigmentation remains in scars.

In the basic group, according to the ultrasound scan results before the treatment, the height of hypertrophic and keloid scars averaged **4.31 mm**; the depth of the atrophic scars was **3.03 mm**.
The presence of not uniform irregular-shaped inclusions with reduced echogenicity in the scar tissue was diagnosed in all the patients; there was strong flattening or absence of a dermo-epidermal junction line.
At the end of the 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).

Similar results have been observed in the control group, treatment performed at the same time, they maintain unimportant small difference in indexes in patients with HS and KS, but they differ significantly in patients with AS (TAB. 1).

Research Groups	Before treatment, mm		After treatment, mm		Changes in 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%

** The differences are significant d < 0,05; ** The differences are significant between the groups.*

TAB. 1

Pathological scars before and after the treatment. Ultrasound scan results.

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

Morphological examination of biopsy materials of the old scar tissue in the basic group before the treatment (100, 200 and 400, hematoxylin eosin stain and Van Gieson's stain): papillary layer of dermis became thin, not full-blown, 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 randomly woven and complete lack of cellular elements (fibroblasts and fibrocytes); these bundles are surrounded by a quite dense 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 the treatment: hyperkeratosic laminated pavement epithelium, flattening of dermo-epidermal line, growth of the 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 the laboratory blood analysis before and after treatment was to study the metabolism of the connective tissue in the process of anti-scars therapy, by the identification of poten-

tial diagnostic indicators of pathological process cicatrization activity.

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 were receiving treatment of pathological scars (TAB. 2).

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 to consider it as a marker, which reflects the catabolism of this protein. About 20% of the 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, which leads to an increased content of easily soluble collagen. Therefore, in patients with impaired metabolism of the connective tissue, the excretion of oxyproline increases in urine, the content of its free fraction in the blood increases, and the content of its bound fraction decreases.

– **GAGs** play an important role in the transport and exchange of water, salts, nutrients, and metabolites in tissues.
The study of the structural and metabolic state of the connective tissue demonstreted high activity of GAGs: the amount was largely increased in the blood plasma of patients in the 1st and in the 2nd group respectively (57,5 ± 2,4 and 49,2 ± 1,3 micromole/l) after our treatment, 1,5 and 1,2 times higher - respectively - than the indexes of the comparison group (conventionally healthy patients).

– Collagenolytic activity (**CLA**) had similar dynamics and didn't depend on the choice of therapeutic method.
The highest CLA values were recorded in patients with the standard therapy method (46,8 ± 2,3 micromole of oxyproline l/h). Levels of plasma CLA in this group exceeded the data of conventionally healthy patients 7-8 times, and in the main group of 4-5 times.

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, to choose the method of therapy.

The average of free oxyproline in both groups before the treatment was 2,17 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±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-bind oxyproline reflects the activity of proliferative

Group and method of treatment	Indexes, M±m							
	free oxyproline millimole/l		bind oxyproline mole/l		GAGs micromole/l		CLA (micromole of oxyproline/l·h)	
	Before the treatment	After the treatment	Before the treatment	After the treatment	Before the treatment	After the treatment	Before the treatment	After the treatment
1st Group (basic) n = 27	1,32±0,8	1,57±0,3**	7,35±1,4	7,7±1,3**	52,7±4,3*	57,5±2,4*	31,7±3,5	39,6±4,2**
2nd 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*
Conventionally 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	

** The differences are significant d < 0,05; ** The differences are significant between the groups.*

TAB. 2

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

processes in the connective stroma of the organs (Kaminskaya G., Puryaeva N.L., 1990; Sodikova N.B, 2002), the indexes of the above mentioned markers in the pathogenesis of the pathological scars formation becomes clear.

The analysis of the research results of these parameters allows us to get to the following conclusions: **the formation of pathological scars is accompanied by profound metabolic disorders of the connective tissue and it is confirmed by a significant increase of collagenolytic activity of blood serum and glycosaminoglycans content in it**.

Evaluative studies 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 the 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, which - on the contrary - reflects the reduction in the intensity of collagen metabolism, perhaps as a result of the therapy we carried out.
The GAGs index may be a predictive criterion for the selection of the 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 the connective tissue is carried out in the intercellular substance under the influence of specific enzymes: collagenase, elastase, proteases, glycosidases.

Peptide chains of collagen are formed 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 the therapy, more than the control group, and in both groups without additional treatment (FIG. 1).

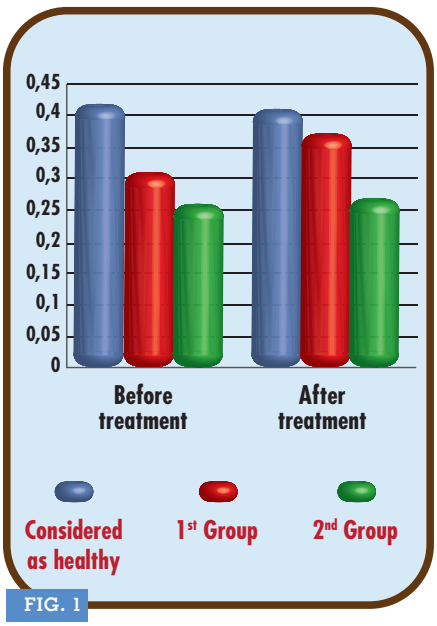


FIG. 1

Vitamin C levels in patients with pathological scars, depending on the treatment method.

Proteins-proteoglycans are called coreproteins. 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 1st group submitted to the basic treatment we saw reaction changes for negative/positive.

In the 2nd group of patients reaction on the core-protein remained unchanged (positive/positive).

Our data indicate **the impact of the treatment on the first reactions of generation of the carbohydrate component of proteoglycans, which occurs in the EPR**.
Most of the subsequent stages of glicosamin chains synthesis 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. To study their effect we measured levels of FAD+ / $FADH_2$ and copper content in the serum of patients before and after therapy.
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 $FADH_2$, as regulators of the mitochondrial respiratory chain.
In all the groups the reduction of $FADH_2$ (0,0294±0.001 micromole/l) by the

Indexes	Standard	1st Group	2 nd 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

** The difference is reliable by comparison with the standard;* *** The difference is reliable between the groups.*

TAB. 3

The concentration of FAD+ and $FADH_2$ in the serum of patients with pathological scars (micromole/l) before the treatment.

control values (0,054±0,005 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 (TAB. 3; FIGG. 2, 3).

– The correlation grows of FAD+/$FADH_2$ lowers the activity of $FADH_2$ 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 $FADH_2$ 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, which leads to a rate reduction of gluconeogenesis and the starting of the pentose phosphate pathway, and consequently, to an increased formation of pathological scars in the second group of patients.
The growth of $FADH_2$ concentration, in comparison with FAD+, slows down the oxidation in the pentose-phosphate pathway, thereby the ratio of lactate/pyruvate is increasing, which further reduces the rate of gluconeogenesis, and increases the lactate concentration in blood.
Oxidative decarboxylation of pyruvate is accompanied by the formation of $FADH_2$ 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+/$FADH_2$ in the cell is a relatively stable rate and that the reduction of $FADH_2$ reduces the speed of decarboxylation of pyruvate. Thus, the rate of change in the ratio of FAD+/$FADH_2$ is an important factor, which represents the energy needed by cells to regulate 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; the tryptophan metabolism is also 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 to influence considerably 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 scientific literature now available.
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 enzyme TDO (tryptophan-2, 3-dioxygenase).
It contributes to the 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 incorpo-

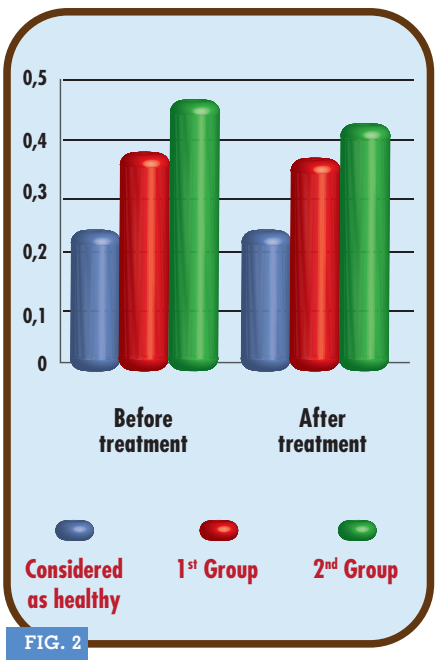


FIG. 2

FAD+ levels in patients with pathological scarring before and after the treatment.

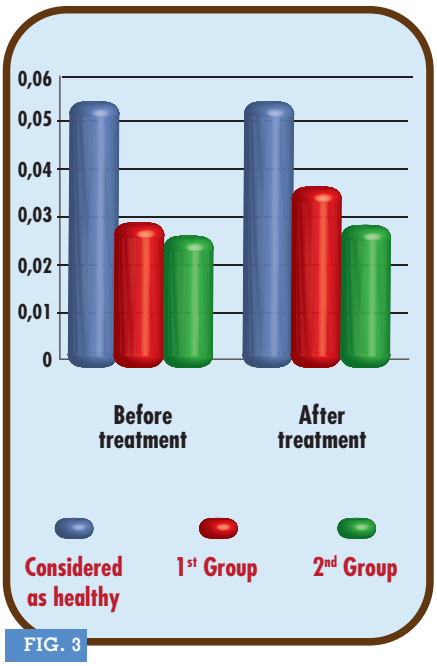


FIG. 3

$FADH_2$ levels in patients with pathological scarring before and after the treatment.

ration of molecular oxygen directly into the molecule of L-tryptophan and its catalyzed reaction is a factor which limites the speed of reaction of the conversion of the substrate.

The examination has found out the activity growth of the TDO and the L-tryptophan content in the serum in both groups before the treatment (TAB. 4).
In these metabolic conditions the way of increased synthesis of NAD^+ and $NADP^+$ coenzyme opens, needed to strengthen 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 to evaluate the reduction of the protein synthesis function of connective tissue in patients with pathological scarring, and particularly about the disturbance of hemoglobin synthesis, which 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.

Indexes	Group of observation, sex	Conventionally 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

** The differences are significant d < 0,05.*

TAB. 4

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

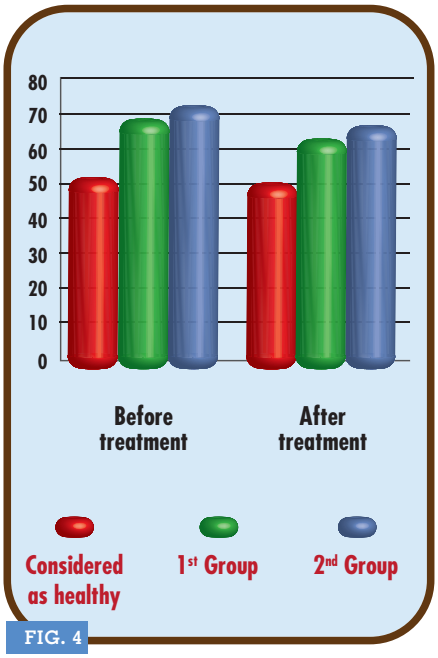


FIG. 4

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

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 reactions of decarboxylation occur (the most part of all the carbon dioxide 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 $NADP \cdot H^+$ 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 it 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 1st group: they were 55,12 ± 2,3 micromole/l and 38,5 ± 2,1 nanomole kynurenine/mg of protein · 1 hour for the L-tryptophan and enzyme TDO activity respectively, and in the 2nd group 59,192 ± 2,5 micromole/l and 40,1 ± 2,1 nanomole kynurenine/mg of protein 1 hour.
Analysis of the metabolic rates dynamics of amino acid L-tryptophan metabolism indicates (FIG. 4) 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 to make a **predictive conclusion about the level of activity or probability of pathological cicatrization**.

Thus, the study of amino acid L-trypto-

phan metabolism makes it possible to confirm objectively the stage of pathological scar development and monitor 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 indexes 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, which has a great prognostic value of the cicatrization process result.

CONCLUSIONS

The results of ths study demonstrate the **high clinical efficacy** of the presented mesotherapeutic treatment of pathological scars compared to the traditional treatment, such as:

1) Reducing treatment time at the average by 93-146 days.
2) Reducing the costs of 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 medicines.
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.
8) Improving the patients' quality of life, and optimization of social rehabilitation.

Mesotherapy of pathological scars with **Made®** and **Guna®-Collagen** can achieve significant functional and aesthetic results in a shorter period of treatment; it doesn't have any complications or negative side effect, nor any special requirements for equipment is needed.
The treatment course is not problematic for the patients' life and work activities, and treatment results to improve the quality of life. ■

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