

# КЛІНІЧНА МЕДИЦИНА

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## DYNAMICS IN CHANGES OF FREE-RADIAL OXIDATION PROTEINS, REGENERATOR PROCESSES, MICROBIAL DISTRIBUTION AND NON-SPECIFIC IMMUNITY IN THE HOMOGENATES OF SCAR TISSUES AT DIFFERENT STAGES OF THE POSTOPERATIVE PERIOD\*

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*The problem of excessive scar formation deserves a particular attention, primarily in the open areas of the human body, because the biochemical mechanisms of the regulation of collagenosynthesis processes after planned surgical interventions of the skin are not well-substantiated. The aim of the study was to optimize the prevention of pathological scars after surgical treatment of congenital neck cysts by determination of the dynamics of changes in biochemical parameters occurring in the neck skin at various stages of healing. It was proved that the combined use of PRF-clot at the intraoperative stage of prophylaxis and ceruloplasmin at the post-operative, in contrast to monotherapy with fibrin membranes, obtained from platelets-rich plasma caused a better functional and aesthetic result, which significantly improved the quality of life of patients in the early and late postoperative periods.*

**Keywords:** scar, congenital neck cyst, prophylaxis.

*Проблема надмірного рубцеутворення, в першу чергу, на відкритих ділянках людського тіла потребує особливої уваги, оскільки біохімічні механізми регуляції процесів колагеносинтезу після планових хірургічних розрізів шкіри є недостатньо обґрунтованим. Метою даної роботи була оптимізація профілактики виникнення патологічних рубців після хірургічного лікування вроджених кіст шиї шляхом визначення динаміки зміни біохімічних показників, що відбуваються в шкірі шиї на різних етапах загоєння. Встановлено, що комбіноване застосування PRF-згустку на інтраопераційному етапі профілактики та церулоплазміну на післяопераційному, на відміну від монотерапії фібриновими мембранами, отриманих із збагаченої тромбоцитами плазми крові, приводить до кращого як функціонального так і естетичного результату, що суттєво покращує якість життя пацієнтів у ранньому та пізньому післяопераційному періодах.*

**Ключові слова:** рубець, вроджені кісти шиї, профілактика.

### Introduction

Scarring is a pathophysiological process of skin regeneration, which is directed at closing of defect. It is an important and relevant problem of modern medicine. The problem of excessive scar formation deserves a particular attention, primarily in the open areas of the human body [1, 2]. Scarring can be caused by various factors, such as traumatic or burn injuries, surgical interventions, purulent inflammatory diseases etc. The regulation of this process depends on many factors, both iatrogenic and general somatic [3, 4].

According to the analysis of literary sources, the study of biochemical mechanisms of the regulation of collagenosynthesis processes after planned surgical interventions of the skin is not well-substantiated [5, 6].

The aim of the study was to optimize the prevention of pathological scars after surgical treatment of congeni-

tal neck cysts by determination of the dynamics of changes in biochemical parameters occurring in the neck skin at various stages of healing.

### Materials and methods

We conducted the biochemical study of the homogenates of the scarred tissues and the intact skin (control group) obtained after surgical excision of scar tissue and skin surpluses during planned surgical interventions at the neck cysts of the embryonic origin.

All patients were divided into 3 clinical groups by the nature of the prophylactic procedure:

Group 1 - 20 patients who received a PRF-clot obtained in a centrifuge in A-PRF test tubes during an operation in 2 layers under the muscle and under the skin.

Group 2 - 20 patients who received a similar intraoperative prophylaxis, but an injection of the drug Biocerulin was performed at the postoperative stage.

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Group 3 (control) - 20 patients, who received a classical technique of intervention without using of prophylactic measures in the postoperative period.

Determination of oxidative modification of blood plasma proteins (OMP) was carried out in conjunction with 2,4-dinitrophenylhydrazine and formation of 2,4-dinitrophenylhydrazones with a typical absorption spectrum. Aldehydogen and ketone derivatives of neutral type were registered at 370 nm (OMP 370), and the base type – at 430 nm (OMP 430) [7]. The activity of superoxide dismutase (SOD) was determined in a supernatant obtained by the method of S. Chevary and co-authors [8]. Determination of the content of reduced glutathione (SH-groups) was performed in the interaction of 5,5'-dithiobis (2-nitrobenzoic) acid (Elman reagent) with SH-groups of the investigated substrate. In this case, thionitrophenyl anion was formed, which was directly proportional to the content of SH-groups [9]. The activity of lysozyme and urease was determined by the bacteriolytic method, which principle was to determine oral dysbiosis for screening of pro- and prebiotics using an enzymatic as-

say [10]. The activity of elastase was determined by the method of Levitsky and co-authors [11]. The evaluation of the intensity of the reparative processes was determined by the amount of RNA and DNA in the homogenate of the skin and scarred tissues according to the A.S. Spirina's method [12].

Statistical analysis of the obtained data was performed using the Statistica 6.0 software package (StatSoft Inc., USA). To verify the statistical significance of the differences in frequency indices, the  $\chi^2$  Pearson correction with Yates correction and Fischer's exact criterion were used.

### Results and discussion

The destruction of proteins is a more reliable marker of oxidative tissue damage than peroxide oxidation of lipids, because the products of oxidative modification of proteins (OMP) are more stable than lipids peroxides, which are rapidly metabolized by peroxidases and low molecular weight of antioxidants.

Table 1. Indices of free radical oxidation of proteins in the skin homogenate at using of PRF-clot

Indices	3 month		6 month		9 month		12 month	
	Control group	Group 1	Control group	Group 1	Control group	Group 1	Control group	Group 1
OMP 370, mmol/g protein	4.09±0.08	2.81±0.07*	2.90±0.04	2.09±0.07*	1.97±0.10	1.72±0.07	1.51±0.05	1.49±0.08
				$p_1 < 0.001$		$p_2 < 0.002$		$p_3 < 0.05$
OMP 430, mmol/g protein	1.85±0.05	1.45±0.03*	1.40±0.08	0.98±0.08*	0.84±0.07	0.72±0.06	0.61±0.05	0.62±0.06
				$p_1 < 0.001$		$p_2 < 0.02$		

The processes of uncontrolled modification of proteins were developed under conditions of oxidative stress and excessive generation of active forms of oxygen (AFO). They had caused protein fragmentation, their denaturation, as well as the formation of primary amino acid radicals, which had entered the secondary interaction

with adjacent amino acid residues. All this factors had created a rather complicated picture of the damaging action of AFO on the protein macromolecule. All this had led to the loss of biological activity of proteins and metabolic disorders, in particular, regenerative processes.

Table 2. Indices of free radical oxidation of proteins in the skin homogenate at using of PRF-clot and Bioceruline

Indices	3 month		6 month		9 month		12 month	
	Control group	Group 2	Control group	Group 2	Control group	Group 2	Control group	Group 2
OMP 370, mmol/g protein	4.09±0.08	2.32±0.06*	2.90±0.04	1.78±0.06*	1.97±0.10	1.54±0.02*	1.51±0.05	1.40±0.06
				$p_1 < 0.001$		$p_2 < 0.002$		$p_3 < 0.05$
OMP 430, mmol/g protein	1.85±0.05	1.18±0.05*	1.40±0.08	0.74±0.05*	0.84±0.07	0.60±0.04*	0.61±0.05	0.56±0.04
				$p_1 < 0.001$		$p_2 < 0.05$		

It was proved that in patients of group 1 the content of 2,4-dinitrophenylhydrazones (that was determined at 370 nm) was significantly reduced by 45.5% after 3 months of observation, after 6 months - by 38.7% and 9 months later - by 14.5%, relative to the data of the control group. The content of 2,4-dinitrophenylhydrazones that was determined at 370 nm was practically not different in patients of the first and third groups, after 12 months of observation. It was evidenced by the literature data. The content of 2,4-dinitrophenylhydrazones that was determined at 370 nm in the skin homogenate of patients with different types of intra- and postoperative prophylaxis of

pathological skin scarring after the surgical treatment of embryonic neck cysts relative to the intact group of patients; the highest rates were found in patients in the control group (after 3 months of observation it was the significant prevalence of 2.8 times, after 6 months - 2 times and after 9 months - 33.0%). The lowest OMP rates were observed in patients of the 2nd group. So, this indicator exceeded the indicator of intact group after 3 months of observation by 60.0%, after 6 months - by 20.0% and after 9 months - by 6.5%.

It has been established that the content of RNA in the first and second experimental groups was significantly

higher, respectively, in 3 (37.3% and 73.0%) and 6 months (11.4% and 20.8%), as compared to the control group of patients ( $p_1 < 0.01$ ). The introduction of PRF-clot during surgical treatment of embryonic neck cysts was

characterized by the highest values of DNA content after 3 months, whereas after 6 months the level of the researched indicator decreased by 17.20%.

Table 3.  
Indices of regenerator process in the skin homogenate at using of PRF-clot

Indices	3 month		6 month		9 month		12 month	
	Control group	Group 1	Control group	Group 1	Control group	Group 1	Control group	Group 1
RNA, mkg/mg	22.15±0.57	30.41±0.26*	26.41±0.57	29.41±0.38*	28.30±0.36	28.45±0.27	27.46±0.35	27.99±0.25
DNA, mkg/mg	17.09±0.27	24.25±0.24*	19.95±0.42	20.08±0.54	20.02±0.27	20.94±0.28	20.47±0.40	21.94±0.45
				$p_1 < 0.01$				

Table 4.  
Indices of regenerator process in the skin homogenate at using of PRF-clot and Bioceruline

Indices	3 month		6 month		9 month		12 month	
	Control group	Group 2	Control group	Group 2	Control group	Group 2	Control group	Group 2
RNA, mkg/mg	22.15±0.57	38.31±0.66*	26.41±0.57	31.90±0.53*	28.30±0.36	29.86±0.38	27.46±0.35	30.04±0.49*
				$p_1 < 0.01$		$p_2 < 0.05$		
DNA, mkg/mg	17.09±0.27	29.92±0.39*	19.95±0.42	26.31±0.32*	20.02±0.27	22.07±0.40*	20.47±0.40	20.62±0.64
				$p_1 < 0.01$		$p_2 < 0.05$		$p_3 < 0.05$

During analysis of changes in the activity of SOD after the surgical treatment of the neck cysts of the embryonic origin relative to the indicators of the intact group of patients, it was noted that the highest rates were found in

patients in the control group (after 3 months of observation, the reliable prevalence was 85.9%, after 6 months - by 35.4% and 9 months later - by 10.2%).

Table 5.  
Indices of system of antioxidant protection in the skin homogenate at using PRF-clot

Indices	3 month		6 month		9 month		12 month	
	Control group	Group 1	Control group	Group 1	Control group	Group 1	Control group	Group 1
SOD, CU.	119.43±4.86	104.60±3.54*	86.96±2.69	80.54±2.41	70.82±2.28	62.97±2.13*	62.97±2.13	59.10±1.42
				$p_1 < 0.001$		$p_2 < 0.001$		
SH-groups, mmol/kg	59.01±2.08	52.83±2.02*	51.92±2.53	49.97±2.02	49.98±2.25	47.35±2.16	49.41±1.93	46.75±1.95

The lowest rates were observed in patients of group 2: after 3 months it was increased by 40.2%, after 6 months - by 12.8% and after 9 months - was not significantly different.

It was determined that the catalase activity after 3 months of observation was prevalence of this indicator by 39.4% ( $p < 0.001$ ), after 6 months - by 12.3% ( $p < 0.05$ ).

Table 6.  
Indices of system of antioxidant protection in the skin homogenate at using of PRF-clot and Bioceruline

Indices	3 month		6 month		9 month		12 month	
	Control group	Group 2	Control group	Group 2	Control group	Group 2	Control group	Group 2
SOD, CU.	119.43±4.86	90.07±2.57*	86.96±2.69	72.45±2.05*	70.82±2.28	60.43±1.52*	62.97±2.13	60.41±2.02
				$p_1 < 0.001$		$p_2 < 0.001$		
SH-groups, mmol/kg	59.01±2.08	49.48±1.76*	51.92±2.53	46.20±2.39	49.98±2.25	45.99±2.08	49.41±1.93	45.30±2.09

The dynamics of changes in the content of SH-groups was less pronounced in terms of the dynamics of enzymatic changes in the antioxidant defense system. In the control group, the prevalence of this indicator was 25.0% ( $p < 0.01$ ) after 3 months of observation; in patients of the first and second groups, this indicator was not significantly different. The activity of elastase after 3 months of observation was fixed significantly lower by 30.9%, after 6 months - by 28.6%, and 9 months later - by 12.1%, relative to data of the third group. The activity of elastase

was not practically different in patients of the second and third groups, after 12 months of observation.

During the study of the dynamics of changes of indices of inflammation, microbial contamination and non-specific immunity, it was noted that the content of lysozyme varied during the observation period. Thus, in patients of group 1 after 6 months of observation this parameter was observed by 21.9% ( $p < 0.001$ ) higher, relative to the data of the previous observation period, after 9 months - by 14.2% ( $p < 0.01$ ), after 12 months - by 4.5%.

Table 7.

Indices of system of inflammation, microbial contamination and non-specific immunity in the skin homogenate at using of PRF-clot

Indices	3 month		6 month		9 month		12 month	
	Control group	Group 1	Control group	Group 1	Control group	Group 1	Control group	Group 1
Elastase, mccat/kg	15.94±0.54	13.58±3.54*	12.19±0.41	10.95±0.30*	9.56±0.36	9.42±0.20	8.89±0.24	8.46±0.30
				p <sub>1</sub> <0.001		p <sub>2</sub> <0.001		p <sub>3</sub> <0.02
Urease, mccat/kg	0.32±0.02	0.22±0.02*	0.17±0.02	0.13±0.01	0.08±0.01	0.06±0.01	0.07±0.02	0.05±0.01
				p <sub>1</sub> <0.001		p <sub>2</sub> <0.001		
lysozyme, U/kg	47.81±1.32	60.54± 1.90*	60.53±2.08	73.81± 2.35*	80.09±3.08	84.32± 2.45	90.22±2.60	88.11±2.85
				p <sub>1</sub> <0.001		p <sub>2</sub> <0.01		

It was found that the activity of the enzyme urease at patient of group 1 after 3 months of observation was significantly decreased by 31.2% relative to the control group, while in the 2nd group - significantly lower by

56.2%, after 6 months - by 52.9 %, and in 9 months - by 50.0%, relative to data of the third group. It had indicated the effectiveness of the proposed method of scar prophylaxis.

Table 8.

Indices of system of inflammation, microbial contamination and non-specific immunity in the skin homogenate at using of PRF-clot and Bioceruline

Indices	3 month		6 month		9 month		12 month	
	Control group	Group 2	Control group	Group 2	Control group	Group 2	Control group	Group 2
Elastase, mccat/kg	15.94±0.54	11.01±0.28	12.19±0.41	8.70±0.26*	9.56±0.36	8.40±0.22*	8.89±0.24	8.24±0.24
				p <sub>1</sub> <0.001				
Urease, mccat/kg	0.32±0.02	0.14±0.01*	0.17±0.02	0.08±0.02*	0.08±0.01	0.04±0.01	0.07±0.02	0.04±0.01
				p <sub>1</sub> <0.02				
lysozyme, U/kg	47.81±1.32	68.57± 2.05*	60.53±2.08	81.64± 2.66*	80.09±3.08	87.08± 2.72	90.22±2.60	91.69±3.08
				p <sub>1</sub> <0.001				

### Conclusions

The analysis of the obtained data suggested that the combined use of PRF-clot at the intraoperative stage of prophylaxis and ceruloplasmin at the post-operative one, in contrast to monotherapy with fibrin membranes, obtained from platelets-rich plasma had a better functional and aesthetic result, which significantly improved the quality of life of patients in the early and late postoperative periods.

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