

## Change of interferons $\alpha$ and $\gamma$ levels in the blood of patients with uveitis as a result of combination therapy with the use of cycloferon, decametoxine electrophoresis and microwave therapy

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**Key words:** uveitis, medicamentous therapy, cycloferon, decametoxine electrophoresis, microwave therapy, interferons  $\alpha$  and  $\gamma$ , blood plasma.

**Introduction.** Searching for new effective pathogenetic approaches to the uveitis treatment is both clinically and socially relevant, considering the pathology high prevalence and disease-induced disability.

**Objective.** To determine the peculiarities of interferons  $\alpha$  and  $\gamma$  expression in the blood of patients with uveitis as a result of combination therapy with the use of cycloferon, decametoxine electrophoresis and microwave therapy.

**Materials and methods.** Patients with endogenous uveitis received a combination therapy with the use of cycloferon, decametoxine electrophoresis and microwave therapy (26 persons). Control groups: cycloferon and decametoxine electrophoresis (20 persons) and cycloferon and microwave therapy (13 persons). The level of interferons  $\alpha$  and  $\gamma$  was measured in the blood plasma and the ophthalmological examination was carried out before and after the treatment. Parametric and nonparametric criteria, as well as correlation analysis, were used in statistical data processing.

**Results.** A significant increase of interferons  $\alpha$  and  $\gamma$  concentration was found as a result of the treatment of patients with uveitis. The increase degree does not depend on the focus of inflammation location and the nature of the clinical course. At the same time, the most significant increases are pronounced the most in severe uveitis. There was found a reliable correlation between the level of interferons before and after the treatment, as well as the clinical characteristics of the disease. The increase of interferons  $\alpha$  and  $\gamma$  level was determined in various clinical forms of uveitis. The most pronounced increase of interferons  $\alpha$  and  $\gamma$  level was detected as a result of decametoxine electrophoresis and microwave therapy on the background of cycloferon administration. It amounts 56.7% and 33.4% respectively.

**Conclusions.** The increase of interferons level was detected in the blood of patients after the treatment. The most significant increase occurred as a result of decametoxine electrophoresis and microwave therapy on the background of cycloferon administration. It may be considered as one of the treatment efficacy criteria.

### Introduction

Endogenous uveitis is a widespread and difficult to treat disease that is often accompanied by complications and disability.

Despite the numerous studies of the pathogenesis of this disease and the understanding of the underlying causes and mechanisms of its progression, the problem of the treatment efficacy remains unsolved [5, 6, 10].

Taking into account the known data on the role of the immune processes violations and the imbalance of pro- and anti-inflammatory cytokines in uveitis progression, the need for the use of immunotropic medications in this disease treatment is beyond doubt [2, 14]. However, when choosing medications the particularities of the immune status of the patient should be taken into account.

Diagnostic significance of the cytokines level evaluation lies in ascertaining the fact of the disease focusing (the increase or the decrease of the concentration) and the severity of these changes in the patient with a particular disease.

Although cytokines are local mediators, in this connection it is expedient to measure their level in the respective tissues or biological fluids. Determination

of cytokines level in the plasma is extremely important because it reflects the general state of the immune system and protective reactions development, characterizing the organism immunoreactivity [11, 12].

Data on the content of cytokines in the peripheral blood can not only characterize the clinical features of the disease, but also the dynamics of the pathological process and the presence (or probability) of exacerbations [13]. It is logical to assume that according to the changing of the cytokine profile one can judge the effectiveness and appropriateness of the therapy.

In order to suppress the autoimmune reactions, it is necessary to use the immunosuppressive agents (corticosteroids, cytostatic agents, cyclosporine A, FK-506). And to eliminate the immunodeficiency disorders it is necessary to use the immunomodulating agents (interferon inducers, activine t, and levamisole).

Wherein it should be remembered that unreasonable and/or excessive immunosuppression or

immunostimulation increases the risk of post-uveitis complications [9, 15].

Optimal, in this context, is the use of immunomodulators (myelopid, imunofan, activine t, levamisole, poludanum, plasmapheresis, hemosorption) weakening the hyperreactivity and / or enhancing reduced immunological indices, that is, normalizing them [8].

Widespread use of new medications in the clinic, anyhow modifying the cytokine status of the organism assumes controlling their level during treatment in order to provide correct cytokine therapy.

Based on the foregoing, the determination of cytokines level, as one of the critical immunopathogenesis factor, in the uveitis treatment dynamics will provide the quantitative data required to monitor the immunocorrective effect of used medications or methods.

**Objective.** Determination of the peculiarities of interferons  $\alpha$  и  $\gamma$  expression in the blood of patients with uveitis as a result of combination therapy with the use of cycloferon, decametoxine electrophoresis and microwave therapy.

**Material and methods**

61 persons were observed and treated against endogenous uveitis in the Department of Inflammatory Eye Pathology of the State Institution "The Filatov Institute of Eye Diseases and Tissue Therapy of the National Academy of Medical Sciences of Ukraine". Their age was between 13 and 76 with an average of  $(38.1 \pm 13.2)$ , including 33 women (54.1%) and 28 (45.9%) men. Distribution of the patients considering the etiological factors was presented in details previously [7]. 17 persons among the patients had anterior uveitis (including 8 with acute iridocyclitis and 9 with chronic iridocyclitis). 41 patients had posterior uveitis (including 19 persons with focal chorioretinitis and 14 with disseminated chorioretinitis and 1 with retinovaskulitis and 7 with optic neuritis). In one case panuveitis was diagnosed.

Patients were examined within standard ophthalmic research protocol, including visometry, tonometry, ophthalmoscopy, visual field.

For the possibility to analyze the efficacy of the proposed combination therapy the patients were divided into three groups:

1st group (20 persons) - patients received cycloferon and decametoxine by electrophoresis;

2nd group (13 persons) - patients received cycloferon and microwave therapy;

3rd group (26 persons) - patients received cycloferon, decametoxine by electrophoresis and microwave therapy.

Cycloferon ("POLYSAN STPF" LLC) was injected intramuscularly by 500 mg, daily scheme (1, 2, 4, 6, 8, 11, 14, 17, 20, 23), 10 injections, total dose 5.0 g.

Decametoxine (CJSC "Darnitsa" Pharmaceutical Company) was used as 0.02% aqueous solution according to the standard procedure of electrophoresis. During transorbital electrophoresis, an active electrode in the form of a tray was poured with 2-3 ml of 2% calcium chloride solution and then with 1 ml of decametoxine. An indifferent electrode was in the neck area. During

intranasal electrophoresis, an active electrode had the form of nasal turunda. In both cases, an anode is positive; the current is increased in stages from 0.3-0.5-0.8 mA to 1 mA, duration of the procedure 3-5-10 minutes. The gap between the two types of electrophoresis - 2 hours.

Microwave therapy was carried out using "Luch-2" apparatus though the contact method in which the electrode is located on the skin of closed eyelids. D'Arsonval currents frequency - 50000 - 100000 fluctuations per second. The exposure is 5-10 minutes and the overall course is 10 sessions.

In the blood plasma of 59 persons the level of  $\alpha$  and  $\gamma$  interferons was determined before and after the treatment by means of solid-phase immune-enzyme analysis (kits of reagents made by "Vector-Best", Novosibirsk, Russia). Units of interferon content (pg/mL) [3, 4].

The obtained data was statistically processed with the use of Statistica 7.0 software and the parametric Student criterion for pair wise comparison of two groups and preliminary assessment of distribution normality, as well as Spearman rank correlation criterion to identify the link between studied parameters [1].

**Results and their discussion**

The results of our previous research [7] have revealed a significant correlation between the clinical characteristics and the features of the pathological process progression and the initial level of interferons  $\alpha$  and  $\gamma$  in the blood of patients with uveitis. Thus, it has been shown that the content of these cytokines is significantly lower in the posterior uveitis and in chronic course of the disease with a high degree of reliability.

The focusing and the intensity of the expression of the abovementioned interferons determination as a result of the treatment in general for the whole group has shown a significant increase in concentration both interferon  $\alpha$ , and interferon  $\gamma$ : by respectively 1.56 and 1.3 times ( $p = 0.0001$  and  $0.0006$ ) (Table 1).

**Table 1.** Interferons level in the blood of patients with uveitis of different localization before and after the treatment

Monitored indicators	Statistical indicators	Terms of research	
		before the treatment	after the treatment
Interferon $\alpha$	n	59	59
	M $\pm$ SD	11.185 $\pm$ 6.602	17.522 $\pm$ 10.660
	p	-	0.000172
Interferon $\gamma$	n	59	59
	M $\pm$ SD	18.386 $\pm$ 8.072	24.188 $\pm$ 9.774
	p	-	0.000627

An analysis of the relevant data, taking into account the localization of the pathological process (Table 2) revealed a similar focusing of changes, which in all cases were reliably significant. It was also shown that the severity of changes does not depend on the location of the disease (interferon  $\alpha$  level after the treatment of the anterior and posterior uveitis has increased in 1.55 and

**Table 2.** Interferons level in the blood of patients with uveitis of different localization before and after the treatment

Uveitis localization	Statistical indicators	Monitored indicators and timelines of research			
		Interferon $\alpha$ (pg/mL)		Interferon $\gamma$ (pg/mL)	
		before the treatment	after	before the treatment	after
Anterior	n	15	15	15	15
	M $\pm$ SD	14.813 $\pm$ 6.419	22.893 $\pm$ 12.007	23.533 $\pm$ 8.880	32.440 $\pm$ 9.916
	p	-	0.029	-	0.015
Posterior	n	44	44	44	44
	M $\pm$ SD	9.948 $\pm$ 6.262	15.691 $\pm$ 9.635	16.632 $\pm$ 7.059	21.375 $\pm$ 8.062
	p	-	0.0013	-	0.0043

**Table 3.** Interferons level in the blood of patients with uveitis of different clinical disease progress before and after the treatment

Uveitis clinical progress	Statistical indicators	Monitored indicators and timelines of research			
		Interferon $\alpha$ (pg/mL)		Interferon $\gamma$ (pg/mL)	
		before the treatment	after	before the treatment	after
Chronic	n	47	47	47	47
	M $\pm$ SD	9.883 $\pm$ 6.053	15.334 $\pm$ 9.173	15.828 $\pm$ 6.180	21.204 $\pm$ 7.364
	p	-	0.001	-	0.0002
Acute	n	12	12	12	12
	M $\pm$ SD	16.283 $\pm$ 6.402	26.092 $\pm$ 12.112	28.408 $\pm$ 6.807	35.875 $\pm$ 9.481
	p	-	0.021	-	0.242

**Table 4.** The level of interferons in the blood of patients with uveitis of different disease severity before and after the treatment

Uveitis severity degree	Statistical indicators	Monitored indicators and timelines of research			
		Interferon $\alpha$ (pg/mL)		Interferon $\gamma$ (pg/mL)	
		before the treatment	after	before the treatment	after
Average	n	53	53	54	53
	M $\pm$ SD	11.132 $\pm$ 6.755	17.338 $\pm$ 10.740	18.157 $\pm$ 7.744	23.647 $\pm$ 9.371
	p	-	0.0004	-	0.0013
Severe	n	5	6	5	6
	M $\pm$ SD	10.200 $\pm$ 4.991	19.150 $\pm$ 10.728	20.860 $\pm$ 11.912	28.967 $\pm$ 12.820
	p	-	0.282	-	0.309

1.58 times ( $p = 0.029$ ), and interferon  $\gamma$  - in 1.38 and 1.29 times, respectively) ( $p = 0.015$ ), i.e., the increase of  $\alpha$  interferon is more pronounced.

Uveitis clinical course particularities (chronic or acute) also do not impact the dynamics indicators of interferon content in the blood of patients as a result of the treatment. In both cases there is interferon  $\alpha$  concentration increase in 1.5 times, and interferon  $\gamma$  in 1.34 and 1.26 times respectively ( $p = 0.0002$ ). These changes are valid except for the data on the increase of interferon  $\gamma$  after the treatment in the group of patients with acute uveitis as a tendency (Table 3).

The most pronounced increase of interferons after the treatment occurs in severe uveitis (Table 4). Thus, the content of interferon  $\alpha$  increases nearly twofold (in 1.88 times), and interferon  $\gamma$  in 1.39 times. However, these changes are not reliable, which can be explained by the small number of observations in the group ( $n = 5$  before the treatment and 6 after the treatment) and sufficiently large spread of individual values in different patients. In persons with an average severity of uveitis the differences between the levels of studied interferons before and after the treatment are reliably relevant and are 56 and 30%, respectively ( $p = 0.0004$  and 0.0013).

Pair correlation analysis data has shown a strong correlation between the levels of studied interferons in patients with uveitis before and after the treatment: correlation coefficient is 0.88 and 0.89 for interferon  $\alpha$  and interferon  $\gamma$ , respectively (Table 5).

**Table 5.** Pair correlation between each studied interferons levels in patients with uveitis before and after the treatment

Statistical indicators	Studied parameters	
	Interferon $\alpha$ (pg/mL)	Interferon $\gamma$ (pg/mL)
n	59	59
r	0.88	0.89
p	<0.05	<0.05

Note: "n" is the number of correlation pairs, "r" is the correlation coefficient; "p" is the level of the differences significance.

The same strong reliable correlation was found between the values characterizing the level of interferons  $\alpha$  and  $\gamma$  before the treatment ( $r = 0.84$ ), as well as their values, achieved as a result of the treatment ( $r = 0.75$ ) (Table 6).

**Table 6.** Pair correlation between interferons  $\alpha$  and  $\gamma$  levels in patients with uveitis before and after the treatment

Statistical indicators	Timelines of research	
	before the treatment	after the treatment
n	59	59
r	0.84	0.75
p	<0.05	<0.05

The data shown in Table 7 indicates the presence of a rank correlation between the clinical characteristics of the disease (uveitis localization and its progression peculiarities), and the level of interferons before and after the treatment.

Characterizing the dynamics of interferons expression as a result of the treatment, taking into account the clinical diagnosis, it should be noted that

the fact of these cytokines level increasing takes place in every known uveitis form, but the changes are reliably significant only for interferon  $\alpha$  in acute iridocyclitis, focal chorioretinitis, optic neuritis, constituting respectively 174, 162 and 153% relative to the corresponding data before the treatment. In other cases, the character of the changes can be classified as a tendency to increase (Table 8).

Analyzing the data that characterizes the level of interferons in the blood of patients with uveitis given the diagnosis (acute and chronic iridocyclitis, disseminated chorioretinitis, retinal vasculitis, optic neuritis, panuveitis) using the Kruskal-Wallis criterion, there was no significant correlation found between the average concentrations of interferon  $\alpha$  both before ( $\chi^2=5.8402$ ,  $df=6$ ,  $p=0.441$ ), and after the treatment ( $\chi^2=7.468$ ,  $df=6$ ,  $p=0.280$ ). Similar data was obtained for interferon  $\gamma$ .

Connection between the average values of this cytokine according to the Kruskal-Wallis criterion before the treatment and after the treatment is not reliable ( $\chi^2=9.230$ ,  $df=6$ ,  $p=0.161$  and  $\chi^2=9.392$ ,  $df=6$ ,  $p=0.153$  respectively). The results obtained with this approach to the statistical experimental data array processing can be explained by the different number of observations in each group, including the isolated retinal vasculitis and panuveitis cases among the observed patients.

To evaluate the electiveness of the proposed combination therapy as the comparison we have representatively marked the groups of patients in which on the top of already administered cycloferon therapy decametoxine electrophoresis, as well as microwave therapy were applied. The obtained results (Table 9) show a positive effect which was evaluated by the degree of interferons level increase in all groups. When comparing the data characterizing the interferons content in the blood after the treatment, it was found that the most pronounced increase in the level of interferons occurs in all the observed groups, but in patients who received the combination therapy (cycloferon + decametoxine electrophoresis + microwave therapy), these changes were reliably significant and constituted 56.7% for interferon  $\alpha$  and 33.4% for interferon  $\gamma$ , respectively, with  $p = 0.009$  and  $0.013$ .

In general the results obtained allow the following conclusions.

**Table 7.** Rank correlation between the level of interferons  $\alpha$  and  $\gamma$  in the blood of patients with uveitis and the disease clinical characteristics

Clinical sign	Monitored indicators	Before the treatment			After the treatment		
		n	r Spearman	p	n	r Spearman	p
Localization	IFN- $\alpha$	59	-0.323486	0.012448	59	-0.256023	0.050321
	IFN- $\gamma$	59	-0.313180	0.015726	59	-0.482378	0.000109
Progress	IFN- $\alpha$	59	-0.374666	0.003460	59	-0.348668	0.006802
	IFN- $\gamma$	59	-0.589786	0.000001	59	-0.524292	0.000020
Severity	IFN- $\alpha$	59	0.095511	0.471779	59	0.075744	0.568562
	IFN- $\gamma$	59	0.108679	0.412587	59	0.103747	0.434240

**Table 8.** Initial level of interferons  $\alpha$  and  $\gamma$  in blood of patients with uveitis considering the clinical diagnosis

Diagnosis	Statistical indicators	Interferon $\alpha$ (pg/mL)		Interferon $\gamma$ (pg/mL)	
		before the treatment	after the treatment	before the treatment	after the treatment
Acute iridocyclitis	n	8	8	8	8
	M $\pm$ SD	16.53 $\pm$ 6.814	28.83 $\pm$ 13.586	25.54 $\pm$ 8.955	34.34 $\pm$ 12.080
	p	-	0.038	-	0.120
Chronic iridocyclitis	n	9	9	9	9
	M $\pm$ SD	11.07 $\pm$ 6.169	14.31 $\pm$ 5.573	19.47 $\pm$ 8.544	27.53 $\pm$ 8.147
	p	-	0.259	-	0.057
Focal chorioretinitis	n	19	19	19	19
	M $\pm$ SD	7.99 $\pm$ 5.478	12.98 $\pm$ 8.238	14.63 $\pm$ 7.329	18.50 $\pm$ 5.644
	p	-	0.034	-	0.077
Disseminated chorioretinitis	n	14	14	14	14
	M $\pm$ SD	12.86 $\pm$ 7.260	20.23 $\pm$ 12.199	18.82 $\pm$ 6.728	24.63 $\pm$ 9.550
	p	-	0.062	-	0.071
Retinovaskulitis	n	1	1	1	1
	M $\pm$ SD	11.600 $\pm$ 0	15.400 $\pm$ 0	16.800 $\pm$ 0	22.200 $\pm$ 0
Optic neuritis	n	7	7	7	7
	M $\pm$ SD	11.59 $\pm$ 5.043	17.74 $\pm$ 4.489	20.21 $\pm$ 5.72	25.33 $\pm$ 8.554
	p	-	0.033	-	0.213
Panuveitis	n	1	1	1	1
	M $\pm$ SD	3.600 $\pm$ 0	4.900 $\pm$ 0	5.500 $\pm$ 0	8.900 $\pm$ 0

**Table 9.** Interferons  $\alpha$  and  $\gamma$  level in the blood of patients with uveitis against the background of different schemes of treatment

Treatment scheme	Statistical indicators	IFN- $\alpha$ (pg/mL)		IFN- $\gamma$ (pg/mL)	
		before the treatment	after the treatment	before the treatment	after the treatment
Cycloferon+decamethoxine electrophoresis	n	20	20	20	20
	M $\pm$ SD	10.73 $\pm$ 6.840	16.37 $\pm$ 9.898	17.02 $\pm$ 8.110	22.45 $\pm$ 9.121
	p	-	0.043	-	0.054
Cycloferon+microwave therapy	n	13	13	13	13
	M $\pm$ SD	9.68 $\pm$ 6.831	15.86 $\pm$ 10.46	17.38 $\pm$ 8.674	22.02 $\pm$ 8.273
	p	-	0.086	-	0.176
Cycloferon+decamethoxine electrophoresis + microwave therapy	n	26	26	26	26
	M $\pm$ SD	12.29 $\pm$ 6.367	19.24 $\pm$ 11.47	19.95 $\pm$ 7.778	26.61 $\pm$ 10.70
	p	-	0.009	-	0.013

**Conclusions**

1. The significant increase in the concentration of interferons  $\alpha$  and  $\gamma$  resulting from the treatment in the whole for the group was shown - in 1.56 and 1.3 times, respectively.

2. The increase degree of the studied interferons level after the treatment does not depend on the localization of the inflammatory focus, however, it was more pronounced for interferon  $\alpha$ , which level has increased

in anterior and posterior uveitis in 1.55 and 1.58 times, whereas interferon  $\gamma$  - in 1.38 and 1.29 times.

3. The character of the clinical course of uveitis (acute or chronic) does not determine the dynamics of the content of interferons in the blood of patients after the treatment (in both cases, concentration interferon- $\alpha$  increases reliably by half, interferon  $\gamma$  in 1.34 and 1.26 times, respectively).

4. The most pronounced interferons increase after the treatment occurs in severe uveitis: interferon  $\alpha$  - in 1.88 times, interferon  $\gamma$  - in 1.39 times; in persons with average severity of uveitis, these differences are respectively 56 and 30%.

5. In patients with uveitis a strong correlation between the level of each researched interferons before and after the treatment was found (the correlation coefficient for interferon  $\alpha$  and  $\gamma$  is respectively 0.88 and 0.89), between the interferons level indicators  $\alpha$  and  $\gamma$  before the treatment ( $r = 0.84$ ), as well as their values, achieved as a result of the treatment ( $r = 0.75$ ).

6. The rank correlation was established between the clinical characteristics of the disease (uveitis localization

and its progress features) and interferons level before and after the treatment.

7. Interferons  $\alpha$  and  $\gamma$  level increase was noted in different clinical forms of uveitis, being reliably significant in acute iridocyclitis, focal chorioretinitis and optic neuritis for interferon  $\alpha$ , constituting 174, 162 and 153 % regarding the data before the treatment.

8. Interferons level increase in the blood of patients after the treatment was found. It was pronounced the most as a result of decamethoxine electrophoresis and microwave therapy under pressure of cycloferon therapy. The changes are reliably significant, constituting for interferon  $\alpha$  and  $\gamma$  56.7 and 33.4%.

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