PROSPECTIVE ASSESSMENT OF CYTOKINES AND REGULATORY PROTEINS CONCENTRATION IN THE TEAR FLUID OF POAG PATIENTS WITH VARIOUS HYPOTENSIVE EFFECTS AFTER NON-PENETRATING DEEP SCLERECTOMY

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ABSTRACT

The aim. *To assess the dynamics of cytokine content in tear fluid of primary open-angle glaucoma (POAG) patients at various terms after non-penetrating deep sclerectomy (NPDS) in relation to the functional state of the outflow tracts.*
Material and methods. *We carried out prospective examination of 65 patients with advanced stage of primary open-angle glaucoma after NPDS. Depending on the course of the postoperative period and the conditions for achieving the hypotensive effect of NPDS, all patients were divided into three groups: group 1 – with the optimal hypotensive effect; group 2 – with the conditional hypotensive effect; group 3 – with no hypotensive effect after NPDS. The intraocular pressure and the concentration of TGF-β, MMP-9, IL-6, IL-8, VEGF-A (121 and 165) in the tear fluid were studied using ELISA method in pre- and postoperative period. We studied the outflow tracts using optical coherence tomography and carried out ultrastructural analysis of filtering blebs tissue.*
Results. *In group 1, the minimum initial concentrations of IL-6, IL-8 and TGF-β in the tear fluid and their moderate increase in the postoperative period; high concentrations of MMP-9 at all stages and an increase in VEGF-A by 2 months after NPDS were registered. In group 2, there was a high level of VEGF-A in the tear fluid before and 2 months after NPDS, an increase in TGF-β, IL-6 and IL-8 in the tear fluid in the early period with their suppression in the late period, as well as an increase in MMP-9 in the early postoperative period. Group 3 had maximum concentrations of TGF-β and IL-8 in the tear fluid initially and in the early postoperative period, suppression of MMP-9 in the tear fluid 2 weeks after and of VEGF-A 2 months after NPDS.*
Conclusion. *Initially high concentrations of IL-6, IL-8, and TGF-β in the tear fluid and the suppression of MMP-9 and VEGF-A in the postoperative period contribute to the surgical failure of the NPDS.*

Key words: *non-penetrating deep sclerectomy, transforming growth factor β, TGF-β, matrix metalloproteinase 9, MMP-9, interleukin 6, IL-6, interleukin 8, IL-8, VEGF-A (121 and 165), cytokines in the tear fluid, extracellular matrix*

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ПРОСПЕКТИВНАЯ ОЦЕНКА КОНЦЕНТРАЦИИ ЦИТОКИНОВ И РЕГУЛЯТОРНЫХ БЕЛКОВ В СЛЁЗНОЙ ЖИДКОСТИ ПАЦИЕНТОВ С ОТКРЫТОУГОЛЬНОЙ ГЛАУКОМОЙ С РАЗЛИЧНЫМ ГИПОТЕНЗИВНЫМ ЭФФЕКТОМ ПОСЛЕ НЕПРОНИКАЮЩЕЙ ГЛУБОКОЙ СКЛЕРЭКТОМИИ

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РЕЗЮМЕ

Цель работы. *Оценить динамику содержания цитокинов в слезе у пациентов с первичной открытоугольной глаукомой (ПОУГ) в различные сроки после непроникающей глубокой склерэктомии (НГСЭ) во взаимосвязи с функциональным состоянием путей оттока.*

Материалы и методы. *Проведено проспективное обследование 65 пациентов с развитой стадией ПОУГ после НГСЭ. В зависимости от течения послеоперационного периода и условий достижения гипотензивного эффекта НГСЭ все пациенты разделены на три группы: группа 1 – с оптимальным гипотензивным эффектом НГСЭ; группа 2 – с условным гипотензивным эффектом НГСЭ; группа 3 – с отсутствием гипотензивного эффекта после НГСЭ. Проводилось исследование внутриглазного давления и концентраций TGF-β, MMP-9, ИЛ-6, ИЛ-8, VEGF-A (121 и 165) в слезе методом иммуноферментного анализа в до- и послеоперационном периоде, а также оптическая когерентная томография путей оттока и ультраструктурный анализ ткани фильтрационных подушек.*

Результаты. *В группе 1 определены минимальные исходные концентрации ИЛ-6, ИЛ-8 и TGF-β слезы и их умеренное повышение в послеоперационном периоде; высокие концентрации MMP-9 на всех этапах и нарастание VEGF-A ко 2-му месяцу после НГСЭ. В группе2 отмечен высокий уровень VEGF-A слезы перед и через 2 месяца после НГСЭ; нарастание TGF-β, ИЛ-6 и ИЛ-8 слезы в раннем периоде с подавлением в позднем, а также повышение MMP-9 в раннем послеоперационном периоде. Для группы 3 характерны максимальные концентрации TGF-β и ИЛ-8 слезы исходно и в раннем послеоперационном периоде, подавление MMP-9 слезы через 2 недели и VEGF-A через 2 месяца после НГСЭ.*

Выводы. *Исходно высокие концентрации ИЛ-6, ИЛ-8, TGF-β в слезе и подавление MMP-9 и VEGF-A в послеоперационном периоде способствуют хирургическому неуспеху НГСЭ.*

Ключевые слова: *непроникающая глубокая склерэктомия, трансформирующий фактор роста β, TGF-β, матриксная металлопротеиназа 9, MMP-9, интерлейкин 6, ИЛ-6, интерлейкин 8, ИЛ-8, VEGF-A (121 и 165), цитокины в слёзной жидкости, внеклеточный матрикс*

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RELEVANCE

In the step-by-step algorithm of primary open-angle glaucoma (POAG) treatment, glaucoma surgery performed to normalize intraocular pressure (IOP) is considered as a step following hypotensive drug therapy in case of its ineffectiveness.

At the same time, the main disadvantage of filtering and fistulizing surgeries is excessive scarring in the area of surgical site, which disrupts the outflow of intraocular fluid (IOF) through the newly created pathways. This process is stimulated by pro-fibrogenic and pro-inflammatory regulatory proteins [1]. Abnormalities in the composition of the anterior chamber aqueous humor of glaucoma patients, in particular an increase in active forms of transforming growth factor β (TGF-β), as well as tumor necrosis factor α (TNF-α), interleukins (ILs) 6 and 8, contribute to fibrogenesis [2, 3]. It is known that a decrease in the activity of matrix metalloproteinases (MMPs) leads to impaired degradation of extracellular matrix (ECM) components [4].

In addition to factors in the anterior chamber aqueous humor, wound processes in the glaucoma surgery region are influenced by regulatory proteins (RPs) and cytokines expressed by conjunctival tissue cells in response to surgical trauma [5]. In addition, subclinical conjunctival inflammation, which is often due to long-term use of some antiglaucoma drugs, is also accompanied by monocyte/macrophage tissue infiltration and expression of growth factors and pro-inflammatory cytokines, predisposing to early filtering bleb (FB) scarring [6, 7].

A number of studies, including the data we obtained and published earlier [8], revealed a correlation between effective IOP reduction after trabeculectomy and non-penetrating deep sclerectomy (NPDS) and the state of the conjunctival lymphatic system. It is assumed, given the data presented, that the negative impact on the outcome of antiglaucoma interventions is caused not only by the initial imbalance of regulatory proteins, but also by the use of antimetabolites during surgery and in the postoperative period that inhibit the growth of both connective tissue and conjunctival lymphatic vessels involved in the outflow of intraocular fluid from FB. Thus, the study by R.A. Bouhenni et al. showed that after intraoperative application of mitomycin C in filtering blebs, a decrease in the density of lymphatic and blood vessels was indicated [9].

Thus, it is relevant in the study of the formation of intraocular fluid outflow pathways after glaucoma surgeries to determine the role of cytokines and regulatory proteins in the structural transformation of the extracellular matrix of filtering blebs and the formation of conjunctival lymphatic vessels.

THE AIM OF THE WORK

To assess the dynamics of cytokines and regulatory proteins content in tear fluid of patients with primary open-angle glaucoma after non-penetrating deep sclerectomy at different stages of the postoperative period in correlation with the functional state of the newly created outflow pathways.

MATERIALS AND METHODS

A prospective examination of 65 patients aged 50 to 70 years (mean age 63.6 ± 4.8 years) with advanced stage POAG and decompensated intraocular pressure (IOP) who underwent NPDS and laser descemetopuncture 14–18 days after the surgery. All operations were performed by the same surgeon and were comparable in terms of intervention volume. Depending on the course of the postoperative period and conditions for achieving hypotensive effect of NPDS, all patients 12 months after surgery were divided into three groups: with optimal hypotensive effect of NPDS (group 1), with conditional hypotensive effect of NPDS (group 2), and with no hypotensive effect of NPDS (group 3).

Group 1included 21 POAG patients (age – 65.4 (53.1; 67.3) years) with IOPg ≤ 16 mmHg; according to optical coherence tomography (OCT) the outflow pathways are functional; FBs are diffuse, according to OCT their content is represented by sparse, hyporeflective extracellular matrix [10]. Based on the results of ultrastructural immunohistochemical examination of tissue samples of functional FPs (n = 4), 5 to 7 lymphatic vessels with steady podoplanin expression were indicated [8]. The postoperative period among group 1 patients was areactive. The patients received standard instillation antibacterial and anti-inflammatory therapy (levofloxacin 0.5 %, dexamethasone 0.1 % and nepafenac 0.1 % in a decreasing order).

Group 2 included 23 patients (age – 63.7 (55.2; 66.8) years) with IOPg ≤ 16 mmHg; according to OCT data, in the early postoperative period the substrate of FBs was sparse ECM with loci of rigid matrix, which in some cases was accompanied by transient IOP elevation. Increased inflammatory reaction of the conjunctiva 2 weeks after surgery was an indication for additional anti-inflammatory and antifibrotic therapy, the detailed description of which is presented below. In the late and delayed postoperative periods in this group a qualified hypotensive effect was achieved. Functional postoperative IOF outflow pathways were formed. According to OCT data, filtration blebs were visualized as widespread hyporeflective subconjunctival structures with sparse ECM.

Group 3 included 21 patients (age – 64.3 (52.2; 67.1) years) with IOPg > 16 mmHg; according to OCT data, changes in the newly created outflow tracts already in the early postoperative period were characterized by the presence of predominantly hyperreflective, rigid ECM. The results of ultrastructural immunohistochemical examination of the tissue of non-functional filtering blebs (n = 8) established the absence of fully developed lymphatic vessels among the studied samples [8]. The postoperative period was characterized by a severe inflam-

matory reaction of the conjunctiva and subconjunctival structures. Despite additional treatment, the hypotensive effect of NPDS was not achieved in this group.

Patients of groups 2 and 3 received a comparable amount of additional anti-inflammatory and cytostatic therapy during the postoperative period, which included subconjunctival injections of corticosteroids and antimetabolites (No. 5), as well as microinvasive needling revisions of the filtering bleb (No. 3) within 2 to 6 weeks after NPDS [11–13].

All studies and manipulations were conducted in compliance with the principles of the World Medical Association Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects.

Patients with POAG were examined before surgical treatment and 2 weeks (early postoperative period), 2 months (late postoperative period), and 12 months (delayed postoperative period) after surgery. The IOP level was determined (ORA, ORA Reichert, USA), the degree of ocular inflammatory reaction was assessed by biomicroscopy, and the state of newly created IOF outflow pathways and reflectivity of extracellular matrix of filtration blebs were determined by OCT (Anterior Segment OCT CASIA2, Tomey, Germany). Patients were divided into 3 groups based on these data. The group with optimal hypotensive effect of NPDS included patients with IOPg ≤ 16 mmHg during the delayed postoperative period without local hypotensive therapy, with functional postoperative IOP outflow pathways according to biomicroscopy and OCT/ultrasound biomicroscopy (UBM), as well as with areactive early postoperative period and no indications for additional anti-inflammatory and antifibrotic therapy.

The criteria of conditional hypotensive effect of NPDS were achievement of IOPg ≤ 16 mmHg in 12 months without local hypotensive therapy with functional outflow pathways according to biomicroscopy and OCT/UBM data, but the hypotensive effect of NPDS was obtained after additional anti-inflammatory and antifibrotic therapy during the early postoperative period.

The criteria for the absence of hypotensive effect of NPDS were as follows: IOPg > 16 mmHg; non-functional or partially functional newly created outflow pathways according to biomicroscopy and OCT/UBM data, despite additional anti-inflammatory and antifibrotic therapy.

Moreover, ultrastructural examination of filtering bleb tissue samples that were obtained during repeated surgical interventions was performed in 12 cases 12–18 months after NPDS. In 8 cases samples of non-functional scar-altered blebs were examined: in 4 cases fragments of functional, leaking filtering blebs were excised due to their significant displacement on the cornea and visual discomfort of the patients. Immunohistochemical staining for DNA expression of cell nuclei (DAPI) and podoplanin, a marker of lymphatic vessel endothelium, was performed with ultrastructural study of the obtained drugs using laser confocal microscope LSM 710 (Carl Zeiss AG, Germany).

The concentration of TGF-β, MMP-9, IL-6, IL-8 and vascular endothelial growth factor A (VEGF-A) (121 and 165) was determined in the tear fluid by ELISA using Human TGF-β, Human MMP-9 ELISA, IL-6-ELISA-Best, IL-8-ELISA-Best and VEGF-ELISA-Best kits (Vector-Best, Novosibirsk). Tear fluid from patients with POAG in the amount of 100 μl was collected capillary method from the lower conjunctival arch 4–6 hours before glaucoma surgery, as well as 2 weeks later (before laser descemetopuncture) and 2 months after NPDS.

Patients were included in the study on a voluntary basis, in accordance with the provisions of the World Medical Association Declaration of Helsinki (1964, rev. 2013). The study was approved by the decision of the Biomedical Ethics Committee of the Scientific Centre for Family Health and Human Reproduction Problems.

Statistical processing of the results of the clinical study was carried out using non-parametric statistics methods with calculation of the Mann-Whitney test. The obtained indicators were considered statistically significant with a significance level of $p < 0.05$. The median (Me) and interquartile range (25th–75th percentiles) were calculated to characterize the scattering in the sample. Taking into account significant deviation in the concentration of the investigated regulatory proteins and cytokines, line graphs of several variables were constructed in Statistica (StatSoft Inc., USA), which allowed us to visualize the dynamics of their content in the tear fluid in the pre- and postoperative period.

RESULTS

Group 1 patients were characterized by minimal (among the comparison groups) initial concentrations of TGF-β in tears and moderate values of this factor 2 weeks and 2 months (in 62 % of cases) after NPDS. However, in 38 % of the cases after 2 months there was a complete suppression of TGF-β tear, probably due to the resolution of postoperative inflammation (Table 1, Fig. 1). Moreover, high concentrations of MMP-9, increase of VEGF-A by the 2nd month after HPDS, as well as minimal (among the comparison groups) concentrations of proinflammatory IL-6 and IL-8 in the early postoperative period and their suppression in the late postoperative period were revealed in group 1 at all stages of the pre- and postoperative period.

Group 2 patients were characterized by extremely high baseline tear VEGF-A level and its peak increase by the 2nd month of the postoperative period. The initial concentrations of TGF-β, IL-6 and IL-8 in tears exceeded the values of group 1, and in the early postoperative period they increased by 1.5–2 times; this was accompanied by an increase in the inflammatory reaction of the conjunctiva 2 weeks after NPDS and was an indication for additional anti-inflammatory and antifibrotic therapy. After 2 months, there was suppression of these factors. Also, patients with conditional hypotensive effect of NPDS were characterized by a signifi-

TABLE 1

REGULATORY PROTEINS CONCENTRATION IN THE TEAR FLUID OF POAG PATIENTS AT VARIOUS STAGES OF THE PERIOPERATIVE PERIOD

Indicators	Group 1, Me (IQR)	Group 1, Me (IQR)	Group 1, Me (IQR)	*p* (Mann – Whitney test)
MMP-9 (baseline), ng/ml	16.1 (6.3–36.3)	15.2 (0.4–28.2)	14.4 (3.18–25.8)	$p_{1-2} > 0.05$ $p_{1-3} > 0.05$ $p_{2-3} > 0.05$
MMP-9 (2 weeks), ng/ ml	112.0 (96.5–142.1)	63.0 (18.5–102.4)	1.7 (0.16–2.2)	$p_{1-2} = 0.001$ $p_{1-3} = 0.001$ $p_{2-3} = 0.001$
MMP-9 (2 months), ng/ ml	25.1 (11.1–57.4)	4.2 (2.04–6.1)	3.6 (0.9–5.9)	$p_{1-2} = 0.001$ $p_{1-3} = 0.001$ $p_{2-3} > 0.05$
TGF-β (baseline), pg/ml	9.1 (0.0–22.0)	36.7 (0.0–96.0)	107.7 (15.7–142.4)	$p_{1-2} > 0.05$ $p_{1-3} = 0.003$ $p_{2-3} = 0.02$
TGF-β (2 weeks), pg/ml	294.6 (189–324.8)	150.4 (104.6–177.6)	590.6 (311.2–669.0)	$p_{1-2} = 0.001$ $p_{1-3} = 0.004$ $p_{2-3} = 0.001$
TGF-β (2 months), pg/ml	153 (0–256.0)	34.4 (0–130.2)	11.7 (0–47.7)	$p_{1-2} > 0.05$ $p_{1-3} = 0.03$ $p_{2-3} > 0.05$
VEGF-A (baseline), pg/ml	504.0 (452.0–572.0)	1018.0 (856.0–1409.0)	617.0 (503.0–709.0)	$p_{1-2} = 0.001$ $p_{1-3} > 0.05$ $p_{2-3} = 0.001$
VEGF-A (2 weeks), pg/ml	703.0 (370.0–847.0)	604.0 (452.0–705.0)	780.0 (624.0–815.0)	$p_{1-2} > 0.05$ $p_{1-3} > 0.05$ $p_{2-3} = 0.001$
VEGF-A (2 months), pg/ml	709.0 (622.0–1180.0)	1015.0 (826.0–1137.0)	439.0 (390.0–437.0)	$p_{1-2} = 0.04$ $p_{1-3} = 0.001$ $p_{2-3} = 0.001$
IL-6 (baseline), pg/ml	23.10 (21.3–25.0)	32.7 (29.3–34.9)	32.3 (30.0–34.4)	$p_{1-2} = 0.001$ $p_{1-3} = 0.001$ $p_{2-3} > 0.05$
IL-6 (2 weeks), pg/ml	55.4 (50.4–72.3)	41.6 (39.6–44.2)	36.8 (22.1–40.1)	$p_{1-2} = 0.001$ $p_{1-3} = 0.001$ $p_{2-3} = 0.003$
IL-6 (2 months), pg/ml	16.2 (11.0–20.6)	17.8 (15.9–19.0)	25.2 (19.3–64.4)	$p_{1-2} > 0.05$ $p_{1-3} = 0.001$ $p_{2-3} = 0.001$
IL-8 (baseline), pg/ml	182.0 (158.3–225.1)	211.6 (181.4–253.4)	461.0 (337.2–649.2)	$p_{1-2} > 0.05$ $p_{1-3} = 0.001$ $p_{2-3} = 0.001$
IL-8 (2 weeks), pg/ml	607.2 (537.0–690.0)	710.4 (607.0–822.6)	919.2 (747.8–1131.0)	$p_{1-2} > 0.05$ $p_{1-3} = 0.001$ $p_{2-3} = 0.006$
IL-8 (2 months), pg/ml	112.0 (82.0–171.0)	84.5 (49.5–118.3)	164.2 (110.9–224.6)	$p_{1-2} = 0.02$ $p_{1-3} = 0.04$ $p_{2-3} = 0.001$

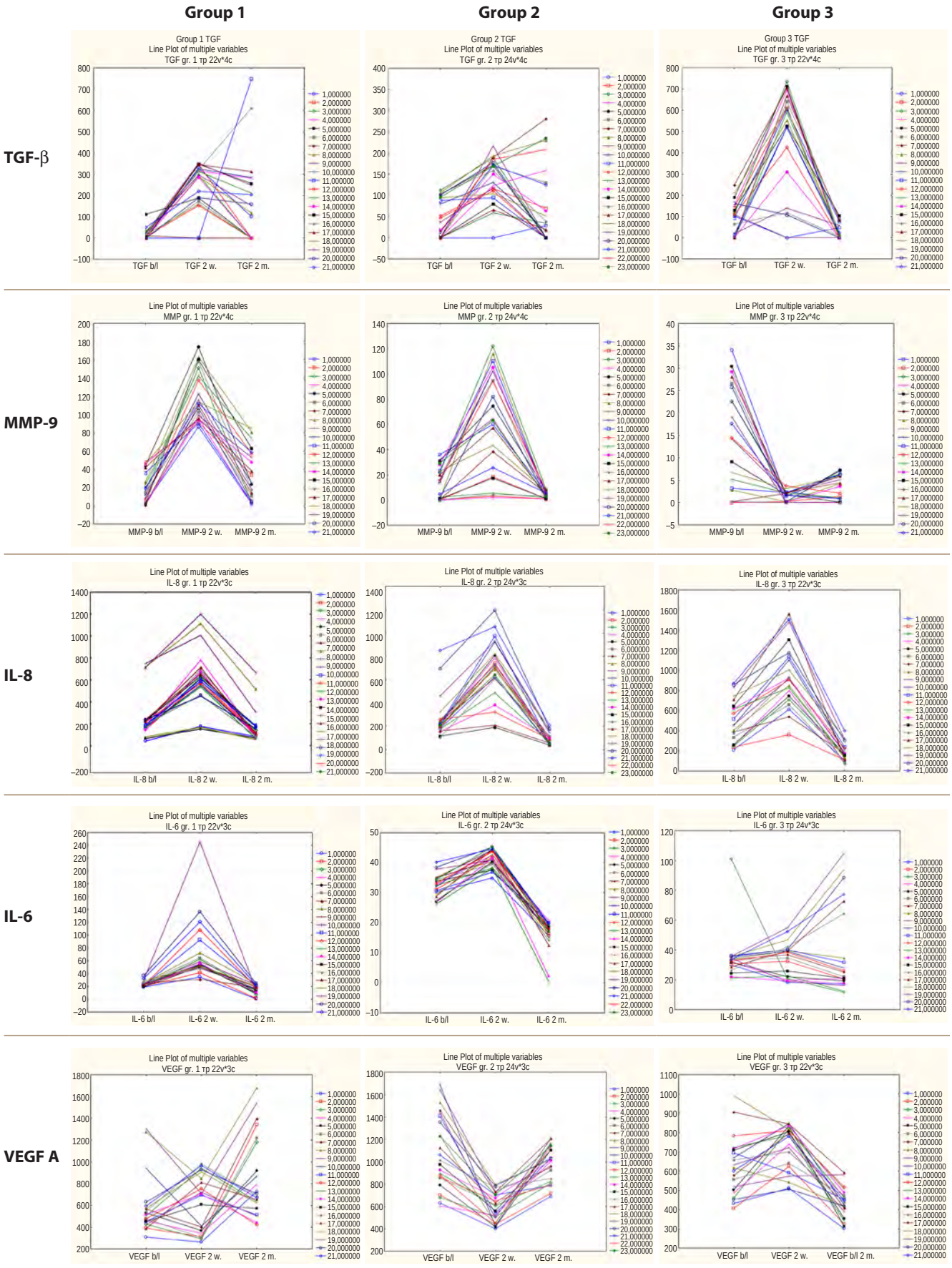

FIG. 1.

Linear graphs of changes in the cytokines and regulatory proteins concentration in the tear fluid at different follow-up periods in POAG patients of three clinical groups

cant increase in tear MMP-9 concentration in the early postoperative period (see Table 1, Fig. 1).

In group 3, tear TGF-β and IL-8 concentrations were maximal both before surgical treatment and in the early postoperative period. The principal difference between groups 1 and 2 was the decrease in tear MMP-9 2 weeks after NPDS. In addition, these patients maintained high IL-6 concentrations and had significant suppression of VEGF-A tears during the late postoperative period.

DISCUSSION

Wound healing is a complex dynamic process that is under constant biochemical control of regulatory molecules that provide specific interactions between cells and components of the extracellular matrix, leading to the restoration of the structural integrity of the tissue. As it was shown earlier [10, 14], functional FB after NPDS is a hypocellular subconjunctival structure in the form of a sparse ECM with fully developed lymphatic vessels, which allows effective outflow of intraocular fluid through the postoperative outflow pathways and determines the persistent hypotensive effect of glaucoma surgery.

The factors predisposing to the formation of optimal hypotensive effect of NPDS are: low initial concentrations of proinflammatory cytokines and regulatory proteins, in particular IL-6, IL-8, TGF-β, which determines minimal inflammatory reaction of the conjunctiva in the early postoperative period; high levels of MMP-9, responsible for timely degradation of the components of the temporary ECM at all stages of the postoperative period, as well as an increase in VEGF-A by the 2nd month after NPDS, which ensures conjunctival lymphangiogenesis.

The significant increase of MMP-9 during the early postoperative period and the peak increase of tear VEGF-A level in the late postoperative period in group 2 may have also contributed to the formation of functional pathways of intraocular fluid outflow, despite the significant inflammatory reaction from the conjunctiva 2 weeks after NPDS. Moreover, the obtained data allowed to establish biological markers of the effectiveness of additional therapeutic measures aimed at controlling the inflammatory reaction and excessive scarring processes in the area of surgical intervention. These are IL-6 and IL-8, as well as TGF-β, suppression of which by the 2nd month of the postoperative period determined the formation of a sufficient (conditional) hypotensive effect of NPDS.

The failure of surgery in group 3 was due to the highest initial level of factors with proinflammatory and profibrogenic activity, increased expression of IL-6, IL-8 and TGF-β in tears in the early postoperative period against the background of a significant decrease in MMP-9, as well as the persistence of high concentrations of IL-6 and suppression of VEGF-A 2 months after NPDS. This determined a severe ocular inflammatory reaction to surgical trauma and its chronization during the delayed postoperative period, active fibrogenesis, impaired degradation of components of the temporary ECM and absence of conjunctival lymphangiogenesis.

CONCLUSIONS

Thus, in the course of this study it was proved that the important conditions for the formation of functional pathways of IOF outflow after NPDS are the preservation of the balance between cytokines and regulatory proteins with proinflammatory and profibrogenic properties, and the factors that ensure timely degradation of temporary ECM components and activation of conjunctival lymphangiogenesis.

Initially high concentrations of IL-6, IL-8 and TGF-β in tear, suppression of MMP-9 and VEGF-A as a result of active and most likely excessive anti-inflammatory and cytostatic therapy in some cases contribute to the surgical failure of glaucoma surgery, as they lead to impaired structural reorganization of the temporary ECM and conjunctival lymphangiogenesis designed to ensure steady intraocular fluid outflow from the filtering blebs.

Conflict of interest

The authors of this article declare the absence of a conflict of interest.

REFERENCES

1. Wynn TA. Mechanisms of fibrosis: Therapeutic translation for fibrotic disease. *Nat Med*. 2012; 18(7): 1028-1040. doi: 10.1038/nm.2807

2. Tripathi RC. Aqueous humor in glaucomatous eyes contains an increased level of TGF-beta 2. *Exp Eye Res*. 1994; 59: 723-727. doi: 10.1006/exer.1994.1158

3. Volkova NV, Malysheva JV, Iureva TN, Kolesnikov SI. The role of biologically active aqueous humor molecules of the anterior chamber and lacrimal fluid in the implementation of the hypotensive effect of non-penetrating deep sclerectomy. *Acta biomedica scientifica*. 2021; 6(2):126-132. (In Russ.). doi: 10.29413/ABS.2021-6.2.14

4. Yamanaka O. Pathobiology of wound healing after glaucoma filtration surgery. *BMC Ophthalmol*. 2015; 15: 157. doi: 10.1186/s12886-015-0134-8

5. Kingsley DM. The TGF-beta superfamily: New members, new receptors, and new genetic tests of function in different organisms. *Genes Dev*. 1994; 8: 133-146. doi: 10.1101/gad.8.2.133

6. Rodrigues ML. Immunohistochemical expression of HLA-DR in the conjunctiva of patients under topical prostaglandin analogs treatment. *J Glaucoma*. 2009; 18: 197-200. doi: 10.1097/IJG.0b013e31818153f4

7. Furtado JM, Paula JS, Soares EG, Dhegaide NH, Rocha EM, Donadi E, et al. Conjunctival inflammation in patients under topi-

cal glaucoma treatment with indication to surgery. *Acta Cir Bras*. 2012; 27: 732-735. doi: 10.1590/s0102-86502012001000011

8. Iureva TN, Malysheva JuV, Klimenkov IV, Sudakov NP. Immunohistochemical identification of lymphatic outflow in filtering blebs after non-penetrating deep sclerectomy (NPDS). *Fyodorov Journal of Ophthalmic Surgery*. 2021; 3: 48-54. (In Russ.). doi: 10.25276/0235-4160-2021-3-48-54

9. Bouhenni RA, Al Jadaan I, Rassavong H, Al Shahwan S, Al Katan H, Dunmire J, et al. Lymphatic and blood vessel density in human conjunctiva after glaucoma filtration surgery. *J Glaucoma*. 2016; 25(1): 35-38. doi: 10.1097/IJG.0000000000000199

10. Iureva TN, Malysheva JV, Kursakova JV, Muskatina EV. Some aspects of filtering bleb formation in patients with primary open-angle glaucoma after non-penetrating deep sclerectomy. *National Journal Glaucoma*. 2022; 21(4): 13-21. (In Russ.). doi: 10.53432/2078-4104-2022-21-4-13-21

11. *Clinical recommendations. Glaucoma, POAG*. 2020. URL: http://avo-portal.ru/doc/fkr/odobrennye-nps-i-utverzhdennye-avo/item/246-glaukoma-otkrytougolnaya [date of access: 23.12.2022]. (In Russ.).

12. Petrov SYu. Modern methods of controlling wound healing after fistulizing glaucoma surgery. Anti-inflammatory drugs and new trends. *Ophthalmology in Russia*. 2017; 14(2): 99-105. (In Russ.). doi: 10.18008/1816-5095-2017-2-99-105

13. Petrov SYu, Safonova DM. Efficacy of bleb needling after trabeculectomy. *Modern Technologies in Ophthalmology*. 2020; 35(4): 142-143. (In Russ.). doi: 10.25276/2312-4911-2020-4-142-143

14. Khoo YJ. Use of trypan blue to assess lymphatic function following trabeculectomy. *Clin Experiment Ophthalmol*. 2019; 47(7): 892-897. doi: 10.1111/ceo.13534

ЛИТЕРАТУРА

1. Wynn TA. Mechanisms of fibrosis: Therapeutic translation for fibrotic disease. *Nat Med*. 2012; 18(7): 1028-1040. doi: 10.1038/nm.2807

2. Tripathi RC. Aqueous humor in glaucomatous eyes contains an increased level of TGF-beta 2. *Exp Eye Res*. 1994; 59: 723-727. doi: 10.1006/exer.1994.1158

3. Волкова Н.В., Малышева Ю.В., Юрьева Т.Н., Колесников С.И. Роль биологически активных молекул влаги передней камеры глаза и слёзной жидкости в реализации гипотензивного эффекта непроникающей глубокой склерэктомии (НГСЭ). *Acta biomedica scientifica*. 2021; 6(2):126-132. doi: 10.29413/ABS.2021-6.2.14

4. Yamanaka O. Pathobiology of wound healing after glaucoma filtration surgery. *BMC Ophthalmol*. 2015; 15: 157. doi: 10.1186/s12886-015-0134-8

5. Kingsley DM. The TGF-beta superfamily: New members, new receptors, and new genetic tests of function in different organisms. *Genes Dev*. 1994; 8: 133-146. doi: 10.1101/gad.8.2.133

6. Rodrigues ML. Immunohistochemical expression of HLA-DR in the conjunctiva of patients under topical prostaglandin analogs treatment. *J Glaucoma*. 2009; 18: 197-200. doi: 10.1097/IJG.0b013e31818153f4

7. Furtado JM, Paula JS, Soares EG, Dhegaide NH, Rocha EM, Donadi E, et al. Conjunctival inflammation in patients under topical glaucoma treatment with indication to surgery. *Acta Cir Bras*. 2012; 27: 732-735. doi: 10.1590/s0102-86502012001000011

8. Юрьева Е.Н., Малышева Ю.В., Клименков И.В., Судаков Н.П. Иммуногистохимическая идентификация лимфатического оттока в фильтрационных подушках после непроникающей глубокой склерэктомии (НГСЭ). *Офтальмохирургия*. 2021; 3: 48-54. doi: 10.25276/0235-4160-2021-3-48-54

9. Bouhenni RA, Al Jadaan I, Rassavong H, Al Shahwan S, Al Katan H, Dunmire J, et al. Lymphatic and blood vessel density in human conjunctiva after glaucoma filtration surgery. *J Glaucoma*. 2016; 25(1): 35-38. doi: 10.1097/IJG.0000000000000199

10. Юрьева Т.Н., Малышева Ю.В., Курсакова Ю.В., Мускатина Е.В. Некоторые аспекты формирования фильтрационных подушек у больных с первичной открытоугольной глаукомой после непроникающей глубокой склерэктомии. *Национальный журнал Глаукома*. 2022; 21(4): 13-21. doi: 10.53432/2078-4104-2022-21-4-13-21

11. *Клинические рекомендации. Глаукома, ПОУГ*. 2020. URL: http://avo-portal.ru/doc/fkr/odobrennye-nps-i-utverzhdennye-avo/item/246-glaukoma-otkrytougolnaya [дата доступа: 23.12.2022].

12. Петров С.Ю. Современная концепция борьбы с избыточным рубцеванием после фистулизирующей хирургии глаукомы. Противовоспалительные препараты и новые тенденции. *Офтальмология*. 2017; 14(2): 99-105. doi: 10.18008/1816-5095-2017-2-99-105

13. Петров С.Ю., Сафонова Д.М. Эффективность нидлинга в пролонгации отдаленного гипотензивного эффекта синустрабекулэктомии. *Современные технологии в офтальмологии*. 2020; 35(4): 142-143. doi: 10.25276/2312-4911-2020-4-142-143

14. Khoo YJ. Use of trypan blue to assess lymphatic function following trabeculectomy. *Clin Experiment Ophthalmol*. 2019; 47(7): 892-897. doi: 10.1111/ceo.13534

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