СОДЕРЖАНИЕ МЕДИАТОРОВ ВРОЖДЕННОГО ИММУНИТЕТА В СЛЕЗНОЙ ЖИДКОСТИ ПАЦИЕНТОВ С СОСУДИСТЫМИ И НЕЙРОДЕГЕНЕРАТИВНЫМИ ПРОЯВЛЕНИЯМИ ДИАБЕТИЧЕСКОЙ РЕТИНОПАТИИ

Ручкин М.П.[1, 2], Маркелова Е.В.[1], Федяшев Г.А.[1, 2]

[1] ФГБОУ ВО «Тихоокеанский государственный медицинский университет» Министерства здравоохранения РФ, г. Владивосток, Россия
[2] ООО «Приморский центр микрохирургии глаза», г. Владивосток, Россия

Резюме. По результатам последних исследований диабетическую ретинопатию можно рассматривать не только как сосудистое заболевание, но и как нейродегенеративный процесс. Изучение состава слезной жидкости используется для оценки состояния локального иммунитета при развитии глазных заболеваний. Однако исследования, изучающие влияние состава слезы при диабетической ретинопатии немногочисленны. Цель исследования – определить уровни IL-1β, IL-10, TGF-β3, MMP-7, TIMP-2, белка S100b, BDNF и NGF в слезной жидкости пациентов сосудистыми и нейродегенеративными проявлениями диабетической ретинопатии. В исследование были включены 80 пациентов с диагнозом сахарный диабет 2 типа, которые были разделены на 2 группы: группа 1 – 40 пациентов, у которых не было клинических признаков диабетической ретинопатии на глазном дне, группа 2 – 40 пациентов с начальными признаками непролиферативной диабетической ретинопатии. Всем включенным в исследование проводилось обследование на оптическом когерентном томографе RTVue-100 (США), определяли объем фокальных потерь ганглиозных клеток сетчатки (FLV). Увеличение FLV выше показателей нормативной базы прибора расценивали как ОКТ-признак нейродегенерации сетчатки. По результатам ОКТ участников первой и второй группы дополнительно разделили на 4 подгруппы: 1А – пациенты без сосудистых изменений на глазном дне и без ОКТ-признаков нейродегенерации сетчатки (n = 12), 1Б – пациенты без сосудистых изменений на глазном дне и с наличием ОКТ-признаков нейродегенерации сетчатки (n = 28), 2А – пациенты с начальной непролиферативной ДР и без ОКТ-признаков нейродегенерации сетчатки (n = 10), 2Б – пациенты с начальной непролиферативной ДР и с ОКТ-признаками нейродегенерации сетчатки (n = 30). Уровень IL-1β, IL-10, TGF-β3, MMP-7, TIMP-2, белка S100b, BDNF и NGF в слезной жидкости определяли с помощью иммуноферментного анализа. Уровни IL-1β и IL-10 в слезной жидкости во всех подгруппах были сопоставимы с контролем на протяжении всего исследования. Содержание TGF-β3 в слезной

Адрес для переписки:
Ручкин Михаил Петрович
ФГБОУ ВО «Тихоокеанский государственный медицинский университет» Министерства здравоохранения РФ
690002, Россия, г. Владивосток, пр. Острякова, 2.
Тел.: 8 (924) 137-04-78.
E-mail: michaelr-n@mail.ru

Address for correspondence:
Mikhail P. Ruchkin
Pacific State Medical University
2 Ostryakov Ave
Vladivostok
690002 Russian Federation
Phone: +7 (924) 137-04-78.
E-mail: michaelr-n@mail.ru

Образец цитирования:
М.П. Ручкин, Е.В. Маркелова, Г.А. Федяшев «Содержание медиаторов врожденного иммунитета в слезной жидкости пациентов с сосудистыми и нейродегенеративными проявлениями диабетической ретинопатии» // Медицинская иммунология, 2023. Т. 25, № 5. С. 1007-1012.
doi: 10.15789/1563-0625-COM-2671

For citation:
M.P. Ruchkin, E.V. Markelova, G.A. Fedyashev "Content of mediators of innate immunity in the tears of patients with vascular and neurodegenerative manifestations of diabetic retinopathy", Medical Immunology (Russia)/Meditsinskaya Immunologiya, 2023, Vol. 25, no. 5, pp. 1007-1012.
doi: 10.15789/1563-0625-COM-2671

DOI: 10.15789/1563-0625-COM-2671

жидкости пациентов группы с начальными признаками непролиферативной ДР (группа 2) было достоверно ($p = 0,001$) ниже в сравнении с контролем и группой 2. Однако отсутствовала достоверная разница ($p > 0,05$) между подгруппами А и Б внутри групп. Концентрация MMP-7 в слезной жидкости во всех подгруппах была достоверно ниже чем в контроле ($p < 0,05$), однако, в подгруппах с ОКТ-признаками нейродегенерации сетчатки (1Б и 2Б) дефицит данной металлопротеиназы был более выражен ($p = 0,0001$). Уровни исследуемых нейропептидов NGF, BDNF и S100B в слезной жидкости не отличались от контроля во всех подгруппах.

Ключевые слова: нейродегенерация, цитокины, нейропептиды, матриксные металлопротеиназы, диабетическая ретинопатия, слеза

CONTENT OF MEDIATORS OF INNATE IMMUNITY IN THE TEARS OF PATIENTS WITH VASCULAR AND NEURODEGENERATIVE MANIFESTATIONS OF DIABETIC RETINOPATHY

Ruchkin M.P.[a, b], Markelova E.V.[a], Fedyashev G.A.[a, b]

[a] *Pacific State Medical University, Vladivostok, Russian Federation*
[b] *Primorsky Eye Microsurgery Center, Vladivostok, Russian Federation*

Abstract. According to the results of recent studies, diabetic retinopathy can be considered not only as a vascular disease, but also as a neurodegenerative process. Study of the composition of the tear fluid is used to assess the state of local immunity in the development of eye diseases. However, studies examining the effect of tear composition in diabetic retinopathy are few. The aim of the study is to determine the levels of IL-1β, IL-10, TGF-β3, MMP-7, TIMP-2, protein S100b, BDNF and NGF in the tear fluid of patients with vascular and neurodegenerative manifestations of diabetic retinopathy. The study included 80 patients diagnosed with type 2 diabetes which were divided into 2 groups: the 1st group included 40 patients who had no clinical signs of diabetic retinopathy on the fundus; the 2nd group included 40 patients with initial signs of non-proliferative diabetic retinopathy. All those included in the study were examined on an optical coherent tomograph RTVue-100 (USA); the volume of focal losses of retinal ganglion cells (FLV) was determined. An increase in FLV above the normative base of the device was regarded as an OCT-sign of retinal neurodegeneration. According to the results of OCT, the participants of the first and second groups were additionally divided into 4 subgroups: 1A – patients without vascular changes in the fundus and without OCT signs of retinal neurodegeneration ($n = 12$); 1B – patients without vascular changes in the fundus and with the presence of OCT signs of retinal neurodegeneration ($n = 28$); 2A – patients with initial non-proliferative DR and without OCT signs of retinal neurodegeneration ($n = 10$); and 2B – patients with initial non-proliferative DR and with OCT signs of retinal neurodegeneration ($n = 30$). The levels of IL-1β, IL-10, TGF-β3, MMP-7, TIMP-2, protein S100 b, BDNF, and NGF in tear fluid were determined by enzyme-linked immunosorbent assay. Levels of IL-1β and IL-10 in tear fluid in all subgroups were comparable to controls throughout the study. TGF-β3 content in the tear fluid of patients in the group with initial signs of non-proliferative DR (group 2) was significantly ($p = 0.001$) lower compared with control and group 2. However, there was no significant difference ($p > 0.05$) between subgroups A and B within groups. The concentration of MMP-7 in the tear fluid in all subgroups was significantly lower than in the control ($p < 0.05$). However, in the subgroups with OCT signs of retinal neurodegeneration (1B and 2B), the deficiency of this metalloproteinase was more pronounced ($p = 0.0001$). The levels of the neuropeptides under study NGF, BDNF and S100 B in tear fluid did not differ from controls in all subgroups.

Keywords: neurodegeneration, cytokines, neuropeptides, matrix metalloproteinases, diabetic retinopathy, tear

Introduction

Currently, the influence of metabolic disorders that occur in diabetes mellitus, on the neurosensory apparatus of the retina, is being actively discussed. According to the results of both our research and a number of studies by other authors, diabetic retinopathy (DR) can be considered not only as a vascular disease, but also as a neurodegenerative process [1, 2]. At the same time, damage to retinal neurons can lead to visible vascular changes and reduced visual function.

In the pathogenesis of the development of retinal neurodegeneration in diabetic retinopathy, one of the basic roles is played by a violation of the balance between damaging and neuroprotective factors [3]. We identified systemic disorders in the levels of cytokines, matrix metalloproteinases and their inhibitors, and neuropeptides in patients with retinal neurodegeneration against the background of type 2 diabetes mellitus, and their role as predictors of the development of this process was also determined.

Study of the composition of the tear fluid is used to determine the effect of local immunity on the development of eye diseases [4]. So the change in the content of various biologically active substances in the tear were found in patients with diabetic retinopathy had a correlation with its severity [5]. However, complex studies studying the composition of tears in diabetic retinopathy are few.

The aim of the study is to determine the levels of IL-1β, IL-10, TGF-β3, MMP-7, TIMP-2, protein S100b, BDNF and NGF in the tear fluid of patients with vascular and neurodegenerative manifestations of diabetic retinopathy.

Materials and methods

The study included 80 patients with an endocrinologist-verified diagnosis of type 2 diabetes mellitus, who, after an ophthalmological examination, were divided into 2 groups: group 1 – 40 patients who did not have clinical signs of diabetic retinopathy on the fundus; and group 2 – 40 patients with initial signs of non-proliferative diabetic retinopathy (the presence of single microhemorrhagia and microaneurysms on the fundus). All participants in the main group took oral hypoglycemic drugs. The average experience of diabetes was 7.5 years. The level of glycated hemoglobin averaged 7.7%. The control group consisted of 30 practically healthy volunteers comparable in sex and age with the main group. Sex distribution in the main group: men 42.5% (n = 34), women 57.5% (n = 46), average age 60.8±6 years. All persons participating in the study provided informed consent. The study was approved by the ethics committee of the Pacific State Medical University (of 16.12.2019 protocol No. 4).

All those included in the study were examined on an optical coherent tomograph RTVue-100 (USA); the volume of focal losses of retinal ganglion cells (FLV) was determined. An increase in FLV above the normative base of the device was regarded as an OCT-sign of retinal neurodegeneration. According to the results of OCT, the participants in the first and second groups were additionally divided into 4 subgroups: 1A – patients without vascular changes in the fundus and without OCT signs of retinal neurodegeneration (n = 12); 1B – patients without vascular changes in the fundus and with the presence of OCT signs of retinal neurodegeneration (n = 28); 2A – patients with initial non-proliferative DR and without OCT signs of retinal neurodegeneration (n = 10); and 2B – patients with initial non-proliferative DR and with OCT signs of retinal neurodegeneration (n = 30).

The levels of IL-1β, IL-10, TGF-β3, MMP-7, TIMP-2, protein S100b, BDNF and NGF in the tear fluid were determined using specific reagents from R&D Diagnostics Inc. (USA) by the sandwich version of the solid-phase enzyme-linked immunosorbent assay, according to the attached instructions. Recording of results was performed using the enzyme-linked immunosorbent assay "Multiscan" (Finland). Quantification of the measured parameters was expressed in pg/mL or ng/mL.

Clinical-instrumental and laboratory examination of patients of the main group was carried out at the initial treatment, as well as after 6 months.

Statistical processing of the results obtained was carried out using the SPSS Statistics 23 program (IBM, USA). Indicators are presented in the form of medians (Me), as well as lower and upper quartiles ($Q_{0.25}$-$Q_{0.75}$). Comparison of quantitative values in unrelated samples was carried out using the Mann–Whitney U test. The Wilcoxon T-test was used in the bound samples. The Spearman rank coefficient was used for correlation analysis. The differences were considered significant at $p \leq 0.05$. The sensitivity and specificity of changes in the studied indicators were assessed by linear regression with the construction of ROC curves.

Results and discussion

The results of a laboratory study of the tear fluid of the study participants are presented in Table 1.

Levels of IL-1β and IL-10 in tear fluid in all subgroups were comparable to controls throughout the study. Given the fact that in other studies serum levels of these cytokines were altered in patients with retinal neurodegeneration in diabetic retinopathy, it is possible that IL-1β and IL-10 play a role. In these

TABLE 1. LEVELS OF THE STUDIED INDICATORS IN THE TEAR FLUID OF THE EXAMINED CONTINGENT, Me ($Q_{0.25}$-$Q_{0.75}$)

Index	Subgroup 1A (n = 12)	Subgroup 1B (n = 28)	Subgroup 2A (n = 10)	Subgroup 2B (n = 30)	Control (n = 30)
IL-1β pg/mL primary	1.62 (1.23-1.95)	1.79 (1.49-2.09)	1.67 (1.39-1.76)	1.86 (1.39-1.98)	1.78 (1.57-2.09)
IL-1β pg/mL after 6 months	1.66 (1.21-1.88)	1.84 (1.49-2.07)	1.79 (1.33-1.72)	1.81 (1.27-2.08)	
IL-10 pg/mL primary	15.21 (10.33-17.85)	15.19 (10.02-18.95)	16.01 (10.32-18.25)	15.68 (10.12-17.71)	16.33 (10.04-18.87)
IL-10 pg/mL after 6 months	16.12 (10.45-18.13)	15.91 (10.65-17.78)	15.89 (10.43-18.93)	16.11 (10.15-18.19)	
TGF-β3 pg/mL primary	102.24 (79.14-131.00)	99.7 (81.38-115.99)	70.82 (37.54-101.50)*	74.8 (35.68-91.87)*	98.49 (84.19-112.35)
TGF-β3 pg/mL after 6 months	103.33 (73.23-119.86)	100.52 (77.14-117.39)	71.89 (38.23-92.6)*	67.33 (34.13-96.34)*	
MMP-7 ng/mL primary	1.88 (1.71-2.66)*	1.34 (1.11-2.16)* #	1.95 (1.82-2.62)*	1.29 (1.15-2.11)* #	2.74 (2.56-2.95)
MMP-7 ng/mL after 6 months	1.86 (1.77-2.65)*	1.38 (1.09-2.11)* #	1.96 (1.77-2.75)*	1.32 (1.13-2.14)* #	
TIMP-2 pg/mL primary	0.15 (0.09-0.23)	0.16 (0.12-0.26)	0.13 (0.07-0.21)	0.17 (0.12-0.24)	0.12 (0.07-0.23)
TIMP-2 pg/mL after 6 months	0.18 (0.12-0.26)	0.13 (0.09-0.23)	0.16 (0.11-0.27)	0.15 (0.09-0.21)	
S100B pg/mL primary	12.55 (3.29-17.56)	11.61 (4.29-16.16)	13.11 (4.33-18.41)	11.65 (5.09-17.33)	13.19 (3.14-17.15)
S100B pg/mL after 6 months	11.53 (3.11-18.42)	11.23 (5.01-16.12)	13.77 (4.11-19.21)	11.37 (5.51-17.94)	
NGF pg/mL primary	8.54 (6.23-11.79)	9.02 (6.23-11.96)	9.13 (6.78-12.03)	9.01 (6.14-11.98)	8.14 (5.99-12.02)
NGF pg/mL after 6 months	8.56 (6.28-12.05)	9.05 (6.25-11.85)	9.16 (6.66-12.05)	9.01 (6.18-12.01)	
BDNF pg/mL primary	10.14 (6.61-15.32)	10.25 (6.98-14.93)	10.64 (7.01-15.33)	10.05 (6.98-15.17)	10.31 (6.91-14.79)

Note. *, significant difference with control group ($p < 0.05$); #, reliable difference between groups A and B within groups ($p < 0.05$).

patients, it would be more indicative in the study of intraocular fluids.

The content of TGF-β3 in the tear fluid of patients in the group with initial signs of non-proliferative DR (group 2) was significantly lower ($p = 0.001$) compared with the control and group 2. However, the presence of OCT signs of retinal neurodegeneration did not affect this indicator, which shows the absence of a significant difference ($p > 0.05$) between subgroups A and B within the groups. TGF-3 β under physiological conditions increases the survival of endothelial cells and pericytes in the vessels of the retina [6]. It is possible that a local decrease in TGF-β3 potentiates the damaging effect of chronic hyperglycemia on retinal vessel cells.

The concentration of MMP-7 in the tear fluid in all subgroups was significantly lower than in the control ($p < 0.05$). However, in the subgroups with OCT signs of retinal neurodegeneration (1B and 2B), the deficiency of this metalloproteinase was more pronounced ($p = 0.0001$). A decrease in the activity of MMP-7 at the systemic and local level in patients with diabetes mellitus was found in many studies [7]. According to some reports, MMP-7 is involved in the activation of some neuroproteins, and a decrease in its activity can lead to a decrease in the neurotrophic support of retinal neurons and potentiates their apoptosis [8, 9].

The content of TIMP-2 in the tear fluid in all subgroups was comparable to control both during the initial examination and after 6 months.

In contrast to the expected results, the levels of the neuropeptides NGF, BDNF and S100B in the tear fluid did not differ from the control in all subgroups. Since these proteins are actively involved in maintaining the vital activity of retinal neurons, it may be more indicative to study their level in intraocular fluids [8].

Correlation analysis showed a significant negative relationship between FLV and the concentration of MMP-7 in the tear fluid ($r = -0.3\ 38$, $p = 0.02$), the level of which was reduced in subgroups with retinal neurodegeneration. However, ROC analysis showed unsatisfactory model quality (AUC = 0, 47) for this indicator as a prognostic marker for the development of retinal neurodegeneration. There was a positive correlation between the levels of MMP-7 and TGF-β3 in the tear fluid ($r = 0.311$, $p = 0.03$).

Conclusion

This study showed a decrease in local levels of MMP-7 and TGF-β3 in the tears of patients with diabetic retinopathy. The presence of vascular symptoms had an effect on the level of TGF-β3. In turn, patients with OCT signs of retinal neurodegeneration showed greater deficiency of MMP-7. Despite the existence of an association between FLV and MMP-7 levels. in the tear, this laboratory indicator for ROC analysis does not have sufficient conjugation with retinal neurodegeneration and cannot be used as a marker of this process.

Thus, further study of the state of the local level of various factors of innate immunity in patients with diabetic retinopathy is required to search for predictors of the development of vascular and neuronal retinal lesions, which will provide a basis for personalized management and treatment of these patients.

References

1. Behl T., Kaur G., Sehgal A., Bhardwaj S., Singh S., Buhas C., Judea-Pusta C., Uivarosan D., Munteanu M.A., Bungau S. Multifaceted role of matrix metalloproteinases in neurodegenerative diseases: Pathophysiological and therapeutic perspectives. *Int. J. Mol. Sci., 2021, Vol. 22, no. 3, pp. 1413-1423.*

2. Neroev V.V., Chesnokova N.B., Pavlenko T.A. Variations of concentrations of angiotensin II, angiotensin-converting enzyme and matrix metalloproteinase-9 in tears and serum of patients with diabetic retinopathy. *Ophthalmology in Russia, 2020, Vol. 17, no. 4, pp. 771-778.* (In Russ.)

3. Poteryaeva O.N., Russkih G.S., Zubova A.V., Gevorgyan M.M., Usynin I.F. Changes in activity of matrix metalloproteinases, concentration of proinsulin and C-peptide in serum depending on the stage compensation of diabetes mellitus. *Bulletin of Experimental Biology and Medicine, 2017, Vol. 164, no. 12, pp. 697-700.* (In Russ.)

4. Ruchkin M.P., Kuvshinova E.R., Fedyashev G.A., Markelova E.V. Neurodegeneration of retina in patients with type 2 diabetes mellitus. *Pacific Medical Journal, 2020, no. 3, pp. 62-64.* (In Russ.)

5. Ruchkin M.P., Markelova E.V., Fedyashev G.A., Yushchuk V.N. Role of cytokines, neuropeptides and matrix metalloproteinases in the immunopathogeneses of retinal neurodegeneration in diabetic retinopathy. *Russian Journal of Immunology, 2022, Vol. 25, no. 4, pp. 515-520.* (In Russ.)

6. Saucedo L., Pfister I.B., Zandi S., Gerhardt C., Garweg J.G. Ocular TGF-β, matrix metalloproteinases, and TIMP-1 increase with the development and progression of diabetic retinopathy in type 2 diabetes mellitus. *Mediators Inflamm., 2021, Vol. 25, no. 2021, 9811361.* doi: 10.1155/2021/9811361.

7. Simo R., Stitt A.W., Gardner T.W. Neurodegeneration in diabetic retinopathy: does it really matter? *Diabetologia, 2018, Vol. 61, no. 9, pp. 1902-1912.*

8. Soni D., Sagar P., Takkar B. Diabetic retinal neurodegeneration as form of diabetic retinopathy. *Int. Ophthalmol., 2021, Vol. 41, no. 9, pp. 3223-3248.*

9. Uzel A.G.T., UGurlu N., Toklu Y., ÇİÇek M., Boral B., Şener B., ÇaĞil N. Relationship between stages of diabetic retinopathy and levels of brain-derived neurotrophic factor in aqueous humor and serum. *Retina, 2020, Vol. 40, no. 1, pp. 121-125.*

Авторы:

Ручкин М.П. *— заочный аспирант кафедры нормальной и патологической физиологии ФГБОУ ВО «Тихоокеанский государственный медицинский университет» Министерства здравоохранения РФ; врач-офтальмолог ООО «Приморский центр микрохирургии глаза», г. Владивосток, Россия*

Маркелова Е.В. *— д.м.н., профессор, заведующая кафедрой нормальной и патологической физиологии ФГБОУ ВО «Тихоокеанский государственный медицинский университет» Министерства здравоохранения РФ, г. Владивосток, Россия*

Федяшев Г.А. *— д.м.н., профессор кафедры офтальмологии и оториноларингологии ФГБОУ ВО «Тихоокеанский государственный медицинский университет» Министерства здравоохранения РФ; главный врач ООО «Приморский центр микрохирургии глаза», г. Владивосток, Россия*

Authors:

Ruchkin M.P., *Postgraduate Student, Department of Normal and Pathological Physiology, Pacific State Medical University; Ophthalmologist, Primorsky Eye Microsurgery Center, Vladivostok, Russian Federation*

Markelova E.V., *PhD, MD (Medicine), Head, Department of Normal and Pathological Physiology, Pacific State Medical University, Vladivostok, Russian Federation*

Fedyashev G.A.*, PhD, MD (Medicine), Professor, Department of Ophthalmology and Otorhinolaryngology, Pacific State Medical University; Head Physician, Primorsky Eye Microsurgery Center, Vladivostok, Russian Federation*

Поступила 21.03.2023
Принята к печати 26.03.2023

Received 21.03.2023
Accepted 26.03.2023