DOI 10 26724/2079-8334-2023-3-85-148-152
UDC 617.735-002-02:616.379-008.64

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EVALUATION OF THE ROLE OF THE RELATIONSHIP OF INFLAMMATORY CYTOKINES WITH THE NEOANGIOGENESIS FACTOR IN THE DEVELOPMENT OF PROLIFERATIVE DIABETIC RETINOPATHY

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A clinical and immunological study was carried out in two groups of patients with diabetes mellitus at the National Center of Ophthalmology named after Academician Zarifa Aliyeva. The first group – 40 patients with non-proliferative diabetic retinopathy; the second group – 42 patients with the development of proliferative diabetic retinopathy within a year. In the first group, there was a significant decrease in inflammatory cytokines and VEGF factor at the local level (VEGF, TNF-α, IL-1β – p=0.001; IL-8 – p<0.001). Diabetic maculopathy and proliferative diabetic retinopathy developed against the background of worsening of the HbA1c diabetes compensation index (9.7±1.1 %; p<0.001) with an increase in angiogenesis factor VEGF and inflammatory cytokines in the blood (VEGF – p=0.034; TNF-α, IL-1β – p=0.001; IL-8 – p<0.001) and tear fluid (VEGF – p=0.018; TNF-α – p=0.005; IL-1β, IL-8 – p<0.001). At the same time, a positive correlation was revealed between the local and systemic levels of VEGF (r=0.333; p=0.031), between the central thickness of the macula and HbA1c, VEGF, TNF-α, IL-1β, IL-8 of a high degree of significance (p<0.001). The results indicate the advisability of a detailed study of the issue of preventive anti-inflammatory therapy along with antiangiogenic treatment for diabetic retinopathy.

Key words: diabetic retinopathy, glycosylated hemoglobin, vascular endothelial growth factor, cytokines, inflammation

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ОЦІНКА РОЛІ ВЗАЄМОЗВ'ЯЗКУ ЦИТОКІНІВ ЗАПАЛЕННЯ З ФАКТОРОМ НЕОАНГІОГЕНЕЗУ У РОЗВИТКУ ПРОЛІФЕРАТИВНОЇ ДІАБЕТИЧНОЇ РЕТИНОПАТІЇ

Клініко-імунологічне дослідження проводилося у двох групах пацієнтів із цукровим діабетом у Національному Центрі Офтальмології імені академіка Зарифи Алієвої. Перша група – 40 пацієнтів із непроліферативною діабетичною ретинопатією; друга група – 42 пацієнти з розвитком проліферативної діабетичної ретинопатії протягом року. У першій групі відзначалося значне зниження цитокінів запалення та VEGF фактора на локальному рівні (VEGF, TNF-α, IL-1β – p=0,001; IL-8 – p<0,001). Діабетична макулопатія та проліферативна діабетична ретинопатія розвивалися на тлі погіршення показника компенсації діабету HbA1C (9,7±1,1 %; p<0,001) зі зростанням фактора ангіогенезу VEGF та цитокінів запалення в крові (VEGF - IL; -1β – p=0,001; IL-8 – p<0,001) та слізної рідини (VEGF – p=0,018; TNF-α – p=0,005; IL-1β, IL-8 – p<0,001). При цьому виявлялася позитивна кореляція між локальним і системним рівнем VEGF (r=0,333; p=0,031), між центральною товщиною макули та HbA1c, VEGF, TNF-α, IL-1β, IL-8 високого ступеня значимості (p<0,001). Отримані результати свідчать про доцільність детального вивчення питання проведення при діабетичній ретинопатії превентивної протизапальної терапії поряд з антиангіогенною.

Ключові слова: діабетична ретинопатія, глікозильований гемоглобін, фактор росту ендотелію судин, цитокіни, запалення.

According to the World Health Organization, the increase in the incidence of diabetes mellitus (DM) globally is projected to reach 366 million people by 2030 [9]. Chronic hyperglycemia, triggering a pathological mechanism of biochemical processes, leads to the development of one of the most severe complications of diabetes – diabetic retinopathy (DR) [4]. Thickening of the vascular basement membrane, proliferation of endothelial cells, and obliteration of capillaries in combination lead to deterioration of retinal microcirculation and hypoxia [8]. Hypoxia-inducible factor-1-alpha (HIF1-a), produced in this case, increases the expression of the vascular endothelial growth factor (VEGF) gene [11].

However, in recent years, much attention has been paid to the role of other biologically active substances (cytokines, growth factors, adhesion molecules) in the pathogenesis of proliferative changes in DR [6]. A large number of publications today highlight the results of studies of various cytokines in blood serum (BS) and biological substrates of the eye (TNF-α, IL-1β, IL-6, IL-8, IL-10, IL-17, IL-4, MCP-1, etc.) during the development of DR [2, 5].

But, despite the large number of multiple studies devoted to the study of the pathogenesis of DR, many questions remain not yet fully resolved and require further detailed consideration. Many aspects of this issue do not have a generally accepted scheme that allows the creation of a model of pathology development, taking into account the participation of all etiopathogenetic factors.

The purpose of this study was a comparative assessment of the level and relationship of angiogenesis factor and inflammatory cytokines (TNF-α, IL-1β, IL-8) at the systemic and local levels in the development of proliferative diabetic retinopathy.

Materials and methods. This retrospective longitudinal study was conducted based on clinical material from the National Center of Ophthalmology named after Academician Zarifa Aliyeva.

82 patients with DM included in the study were divided into two groups. At the initial treatment of all patients, NPDR was diagnosed. The first group (I) included 40 patients without signs of the development of a proliferative process; the second (II) included 42 patients with the transition from NPDR to PDR within a year. In group II, at 6 months, only 36 % of 42 patients remained in the non-proliferative stage of DR, while 64 % (27/42) progressed from NPDR to PDR. After one year in group II, 100 % of patients (42/42) had progressed to PDR. Exclusion criteria were any other pathology of the retina (except for NPDR), active inflammation of the ocular adnexa, clouding of the optical media of the eye, intraocular surgery or laser intervention within the last 3 months, autoimmune systemic diseases, and oncological diseases. The groups did not differ statistically in age, gender, or mean duration of diabetes. The mean age of patients in the two groups was 57.5±8.8 and 56.0±10.5 years (p=0.581), male composition – 45 % (18/40) and 52 % (22/42) (p= 0.507), the mean duration of diabetes was 12.0±5.8 and 13.5±4.2 years (p=0.098), respectively. Although there were more patients with type I diabetes in group II (52 % – 22/42) than in group I (40 % – 16/40), there was also no significant difference between the groups based on the type of diabetes (p=0.264). Also in group II, the relative number of patients suffering from concomitant hypertension (71 % – 30/42), coronary heart disease (31 % – 13/42), nephropathy (41 % – 17/42) and neuropathy (57 % – 24/42) was higher than in group I (60 % – 24/40, 23 % – 9/40, 25 % – 10/40, 55 % – 22/40) accordingly, but this difference was not significant (p=0.278, p=0.391, p=0.138, p=0.846).

All patients provided written informed consent to participate in the study. The study was approved by the Azerbaijan Medical University Ethics Committee (protocol No. 25) and was conducted per the Declaration of Helsinki.

Patients underwent ophthalmological and laboratory examinations. Ophthalmological research methods: determination of best corrected visual acuity (BCVA) (Huvitz Chart Projector CCP-3100 (HUVITZ Co, LTD, South Korea), biomicroscopy of the anterior segment of the eye (slit lamp TOMEY TSL-5000, TOMEY, Japan), tonometry (non-contact tonometer FT-1000, TOMEY, Japan), fundus ophthalmoscopy (slit lamp TOMEY TSL-5000, TOMEY, Japan with Ocular High Mag 78D lens, Ocular Instruments Inc., USA), optical coherence tomography (OCT) (Cirrus optical coherence tomography HD-OCT 5000, Carl Zeiss Meditec AG, Germany), fluorescent angiography (FAG) (Carl Zeiss FF450, Germany).

An endocrinologist assessed the general condition of all patients and monitored diabetes indices. The glycosylated haemoglobin level in the blood (Glycosylated hemoglobin – HbA1c) was determined using affinity chromatography. The laboratory examination also included determination, using the ELISA method, of the level in blood serum (BS) and tear fluid (TF) of VEGF, cytokines TNF-α, IL-1β, and IL-8.

The results were processed by the method of variation statistics with the calculation of arithmetic means, standard deviation, standard error, and minimum and maximum sample values. A comparative assessment between the parameters of the two groups was carried out using the Mann-Whitney U-test (Pu), with the initial indices in the corresponding group using the Wilcoxon W-test (Pw). The statistical significance of differences was assessed at $p<0.05$. The Spearman criterion (Rho) calculated the correlation between the parameters. Statistical analysis of the results was carried out using MS Excel software.

Results of the study and their discussion. The mean level of the HbA1c diabetes compensation index at the initial treatment of patients was higher than the reference values in both groups (in group I – 7.8±1.5 %, in group II – 8.9±1.2 %). In contrast, in group II, it was also significantly higher than the indicator of the first group (p=0.001). After a year, the mean HbA1c level in group I does not change significantly (7.8±1.2 % – p=0.903), while in group II with progression of NPDR, this parameter is significantly higher than the initial values (9.7±1.1 %) and values in group I (p<0.001).

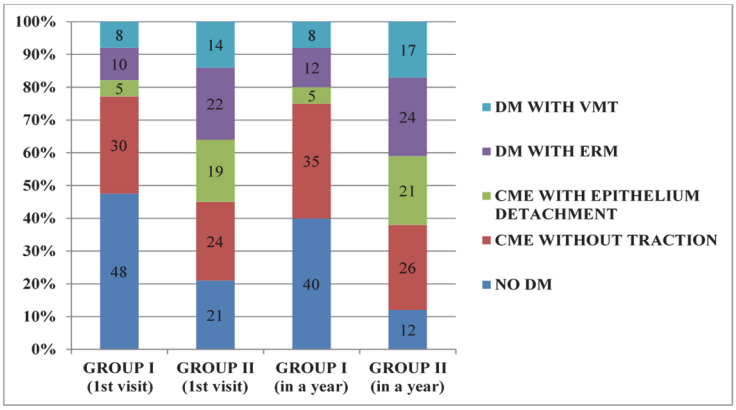


Fig. 1. Distribution of patients in the studied groups based on the presence of DM at the initial visit of patients and after a year (in %)

In both groups, various forms of diabetic maculopathy (DM) were recorded at the initial presentation (Fig. 1).

In group I, there were significantly fewer patients with DM initially – 53 % (21/40), than in group II – 79 % (33/42) (p=0.004). The most common form of DM in both groups was cystoid macular edema (CME) without traction – in 30 % of patients (12/40) and 24 % (10/42), respectively, in the first and second groups. A year later, despite the increase in the number of patients with DM in both groups, their relative number in group II (88 % – 37/42) remained higher than the same parameter in group I (60 % – 24/40).

Clinical parameters of deterioration of the macular fundus and the progression of DR in the second group of patients were confirmed by objective indices of visometry and OCT. During the year, there were no significant changes in the mean BCVA values in group I (p = 0.383), while in group II, BCVA was initially significantly less than in the first group I – 0.18 ± 0.19 (p = 0. 05), during the year it significantly decreased from 0.18±0.19 to 0.12±0.09 (p=0.001). Accordingly, in the second group at the initial treatment, the mean indices of the state of the macular region – the central macula thickness (CMT) and the total macula volume (TMV) – were significantly higher than similar indices in group I: 393.2±168.0 μm and 437.9±145.1 μm (p=0.042); 11.9±2.3 mm³ and 12.9±2.2 mm³ (p=0.034). After a year, the mean CMT and TMV indices in group I significantly decreased to 256.5±23.2 μm and 9.9±1.0 mm³, respectively (p<0.001). In group II with the progression of NPDR, the mean CMT (424.7±133.0 μm) and TMV (12.9±1.9 mm³) remained significantly higher than in group I (p<0.001), did not significantly change (p=0.817, p=0.288) relative to the primary values.

Table 1 presents comparative results of a study of the content of the angiogenesis factor VEGF and key inflammatory cytokines in the BS in both groups of patients at their initial treatment and after a year.

Table 1

Results of comparative assessment of cytokines in BS (pg/mL)

Research Factor	Study time	I (n=40)	II (n=42)	Pu
VEGF	First visit	243.0±19.8 (101.7–548.8)	333.6±18.3 (178.4–558.9)	0.001
	In a year	230.4±17.1 (112.4–509.4)	344.2±19.1 (164.6–589.4)	<0.001
	Pw	0.214	0.034	
TNF-α	First visit	5.3±0.5 (0.3–12.2)	7.7±0.5 (0.9–13.4)	0.001
	In a year	5.3±0.4 (0.7–11.1)	8.4±0.4 (3.2–13.1)	<0.001
	Pw	0.667	0.001	
IL-1β	First visit	7.1±0.5 (1.2–15.9)	12.1±0.6 (3.2–18.5)	<0.001
	In a year	7.3±0.5 (2.9–15.6)	12.9±0.5 (6.3–20.3)	<0.001
	Pw	0.393	0.001	
IL-8	First visit	22.6±2.5 (0.4–61.8)	38.0±2.3 (4.3–63.2)	<0.001
	In a year	23.0±2.2 (1.9–54.2)	40.5±2.1 (9.8–64.7)	<0.001
	Pw	0.609	<0.001	

As a result of a comparative assessment of the angiogenesis factor VEGF and inflammatory cytokines TNF-α, IL-1β, and IL-8 in the BS, it was found that in group II, both at the initial visit of patients and a year later with progression of NPDR, the mean level of the studied factors was significantly higher relative to the parameters in group I without progression of NPDR (p=0.001; p<0.001). A year later, there were no significant changes in the mean level of inflammatory cytokines and VEGF factor in group I without the development of PDR, while in group II, the results of a significant increase in all studied factors were obtained (VEGF – p=0.034; TNF-α, IL-1β – p=0.001; IL-8 – p<0.001).

Table 2

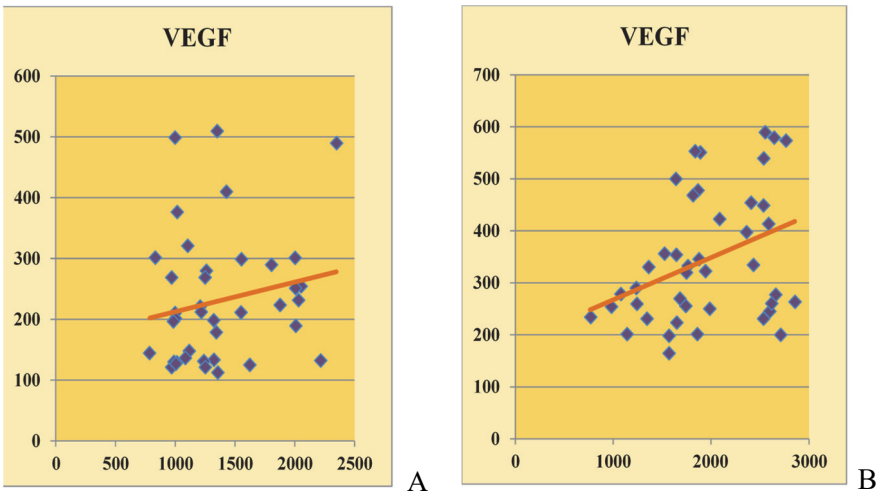
Results of comparative evaluation of cytokines in TF (pg/mL)

Study Factor	Study time	I (n=40)	II (n=42)	Pu
VEGF	First visit	1657.5±96.4 (765–2987)	1869.7±89.7 (778–2742)	0.090
	In a year	1367.9±65.9 (786–2348)	1944.5±86.4 (765–2856)	<0.001
	Pw	0.001	0.018	
TNF-α	First visit	28.9± 1.4 (17.5–48.3)	31.4± 1.4 (16.4–48.9)	0.207
	In a year	25.5± 1.0 (16.8–42.1)	33.1± 1.3 (18.7–50.2)	<0.001
	Pw	0.001	0.005	
IL-1β	First visit	27.0± 1.6 (11.7–44.2)	24.6± 1.4 (9.6–41.5)	0.278
	In a year	23.0± 1.2 (12.8–40.1)	28.0± 1.4 (12.9–48.9)	0.009
	Pw	0.001	<0.001	
IL-8	First visit	19.8±2.2 (3.4–46.8)	19.2± 1.9 (4.1–49.4)	0.967
	In a year	14.9± 1.6 (3.2–42.6)	26.3± 2.5 (5.6–66.7)	0.001
	Pw	<0.001	<0.001	

Table 2 presents the results of a comparative study of the content of the angiogenesis factor VEGF and inflammatory cytokines in the TF in both groups of patients at their initial treatment and a year later.

In contrast to systemic indices, a comparative assessment of the studied factors in the TF revealed that the mean level of VEGF factor and inflammatory cytokines did not have a significant difference between the two groups during the initial treatment of patients. But a year later, with the progression of NPDR in group II, there is an increase in the local level of VEGF factor and inflammatory cytokines, which is significantly different from both the indices in group I and the primary indices (VEGF – $p = 0.018$; TNF-α – $p = 0.005$; IL-1β, IL-8 – $p<0.001$). In addition, in group I, on the contrary, there was a significant decrease in the mean level of the studied factors at the local level (VEGF, TNF-α, IL-1β – $p=0.001$; IL-8 – $p<0.001$).

For a more detailed representation of the relationship between the local and systemic levels of the main angiogenesis factor VEGF, which plays a key role in the etiopathogenesis of proliferation during the progression of NPDR, it was of interest to calculate the correlation coefficient between these indices. As the results showed, a moderate positive correlation was established between the local and systemic VEGF levels at the initial treatment of patients in both groups (group I – $r=0.55$; $p<0.001$; group II – $r=0.335$; $p=0.03$). The results of calculating the correlation coefficient between these indices in a year are shown in Fig. 2.

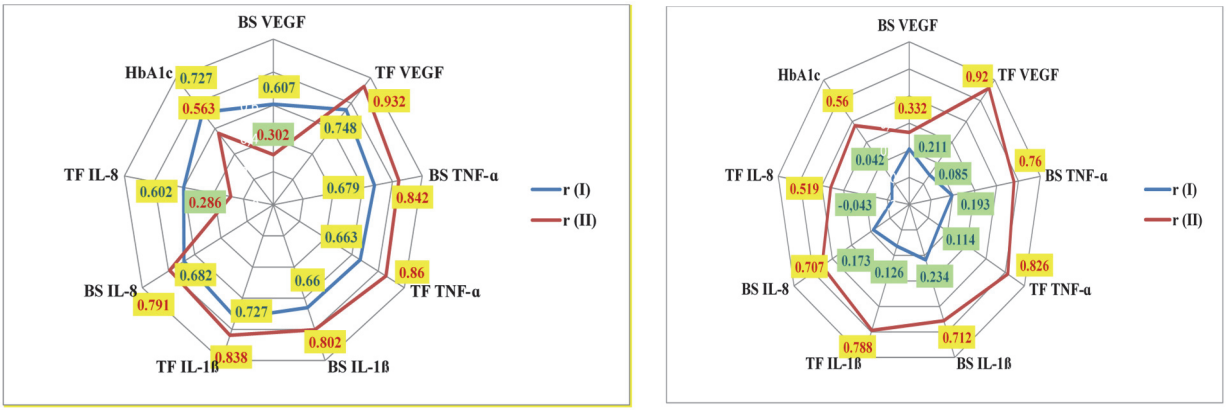


A B

Fig. 2. Correlation between VEGF in the TF and VEGF in the BS when patients re-apply a year later (Spearman's rank coefficient). A – in group I ($r=0.188$; $p=0.246$); B – in group II ($r=0.333$; $p=0.031$)

Interestingly, after a year, the significant significance of the moderate positive correlation between the local and systemic levels of the angiogenesis factor VEGF remained only in group II ($r=0.333$; $p=0.031$). In group I, the relationship between these indices was unreliable ($r=0.188$; $p=0.246$).

The feasibility of a comparative calculation of the correlation between CMT and laboratory parameters at the initial visit of patients and after a year is presented in Fig. 3.



A B

Fig. 3. Correlation between CMT and laboratory parameters (Spearman's rank coefficient) at the initial visit of patients (A) and after a year (B). Indices of the Spearman coefficient in group I are marked in blue and in group II – in red. Positive correlation indices with a high degree of significance ($p<0.001$) are shaded in yellow, while those without significant significance are shaded in green.

In group I, at initial treatment, a strong positive correlation of a high degree of significance was revealed between CMT and HbA1c ($r= 0.727$; $p<0.001$), VEGF factor in the TF ($r= 0.748$; $p<0.001$), IL-1ß in the TF ($r= 0.727$; $p<0.001$). With other laboratory indices at the systemic and local level, CMT's degree of positive correlation in group I was of moderately high significance.

In group II, at the initial treatment, the correlation coefficient between CMT and VEGF in the BS ($r=0.302$; $p=0.052$) and IL-8 in the TF ($r=0.286$; $p=0.066$) was insignificant. A strong positive relationship

was established with VEGF in the TF and other cytokines at the systemic and local level, with HbA1c a moderate (r= 0.563; p<0.001) positive relationship with CMT of a high degree of significance (p<0.001).

After a year in group I without DR progression, the correlation coefficients between CMT and laboratory parameters were insignificant. In group II, with the development of PDR, CMT had a moderate and strong positive relationship with all study factors at the systemic and local level of a high degree of significance (p<0.001).

The role of the relationship between the imbalance of pro- and anti-inflammatory cytokines and the dynamics of changes in vasoproliferative factors in the pathogenesis of DR is currently being discussed from various positions. Literature data indicate the diagnostic value of the increase in TNF-α concentration in BS, the relationship between the concentrations of VEGF and TNF-α, IL-1β in BS and the severity of the disease [3, 7], the role of IL-8 as a marker of ischemic inflammatory response and visual impairment in the progression of PDR and the development of diabetic macular edema [10]. Inflammatory cytokines link the key links in the pathogenesis of DR in the early stages and during the development of proliferative complications [2, 5]. Our results allow us to conclude the prognostic significance of the interrelated increase in the angiogenesis factor VEGF and inflammatory cytokines (VEGF, TNF-α, IL-1β, IL-8) at the systemic level in the development of DR. Further, with the progression of NPDR to PDR, the interdependent growth of these factors at the systemic and local levels determines the important role of inflammation in triggering a cascade of processes leading to pathological proliferation. Along with antiangiogenic therapy, which is widely used today in the treatment of DR [1], it is advisable to consider the need for preventive anti-inflammatory therapy to prevent the worsening of diabetic damage to the organ of vision.

Conclusions

1. In the absence of proliferative changes in NPDR and stable HbA1c values (7.8±1.2 %; p=0.903), there was a significant decrease in the mean level of inflammatory cytokines and VEGF factor at the local level (VEGF, TNF-α, IL-1β – p =0.001; IL-8 – p<0.001).

2. DM and PDR developed against the background of worsening of the HbA1c diabetes compensation index (9.7±1.1 %; p<0.001) with an increase in the values of VEGF angiogenesis factor and inflammatory cytokines in the BS (VEGF – p=0.034; TNF-α, IL-1β – p=0.001; IL-8 – p<0.001) and TF (VEGF – p=0.018; TNF-α – p=0.005; IL-1β, IL-8 – p<0.001).

3. With the development of proliferative changes in DR and damage to the macular area, a positive correlation was revealed between the local and systemic levels of VEGF (r=0.333; p=0.031), between CMT and HbA1c, VEGF, TNF-α, IL-1β, IL-8 in the BS and the TF of high significance (p<0.001), which was not observed in patients without the development of proliferative complications.

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Стаття надійшла 22.07.2022 р.