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Diabetic retinopathy as a subclinical inflammation: Vitreous and serum cytokine analysis

Diabetična retinopatija kot subklinično vnetje: Analiza citokinov v steklovini in serumu

Mojca Urbančič,[1] Peter Korošec,[2] MatjažFležar,[2] Mojca Globočnik Petrovič[1]

[1] Department of Ophthalmology, University Medical Centre Ljubljana, Ljubljana, Slovenia
[2] University Clinic of Respiratory and Allergic Diseases Golnik, Golnik, Slovenia

Correspondence/ Korespondenca:
Mojca Urbančič, e: mojca.urbancic@kclj.si

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Abstract

Background: Diabetic retinopathy can be defined as chronic subclinical inflammation. The purpose of this study was to investigate levels of inflammatory cytokines in the vitreous and serum in patients with proliferative diabetic retinopathy (PDR) and in the control group. In addition, the purpose was to evaluate the levels of cytokines with regard to the activity of PDR. A better understanding of intraocular inflammation in patients with PDR could lead to the development of new treatment options for these patients.

Methods: The study included 37 patients with PDR (37 eyes) that required vitrectomy. Twenty patients with idiopathic macular hole (MH) served as a control group. The activity of PDR was defined based on preoperative examination and intraoperative evaluation of the fundus. Cytometric Bead Array (CBA) method was used for cytokine analysis. Cytokines interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), macrophage inflammatory protein-1α (MIP-1α), macrophage inflammatory protein-1β (MIP-1β), monocyte chemoattractant protein-1 (MCP-1), vascular endothelial growth factor (VEGF), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10) and interleukin-12 (IL-12) were analysed in vitreous and serum samples.

Results: Levels of all cytokines in the vitreous differed significantly from levels in the serum. Levels of MCP-1, VEGF, IL-6, IL-8 were significantly higher in the vitreous of patients with PDR in comparison with vitreous levels in the control group. Vitreous levels of MIP-1α, IL-10 and IL-12 in patients with PDR were significantly lower in comparison with the control group ($p < 0.05$). Serum levels of cytokines were similar in both groups. Patients with active PDR had higher vitreous levels of MCP-1, VEGF, IL-6 and IL-8 in comparison with patients with inactive disease, although the differences were not significant.

Conclusion: Significant differences in cytokine levels in the vitreous compared to serum levels in patients with PDR indicate the importance of local intraocular inflammation. Higher levels of MCP-1, VEGF, IL-6 and IL-8 might reflect the activity of PDR.

Izvleček

Izhodišče: Diabetično retinopatijo lahko opredelimo kot kronično subklinično vnetje. Želeli smo ugotoviti razlike v ravneh vnetnih citokinov med steklovino in serumom pri bolnikih s proliferativno diabetično retinopatijo (PDR) in pri kontrolni skupini ter primerjati ravni citokinov v steklovini glede na aktivnost PDR. Boljše poznavanje vnetnega dogajanja v očesu bolnikov s PDR bi lahko prispevalo k razvoju novih načinov zdravljenja teh bolnikov.

Metode: V raziskavo smo vključili 37 bolnikov (37 oči) s PDR, pri katerih je bila potrebna vitrektomija, ter 20 bolnikov (20 oči) z idiopatskim foramnom makule (FM), ki so predstavljali kontrolno skupino. Glede na pregled pred operacijo in oceno očesnega ozadja med samim posegom smo opredelili aktivnost PDR. V steklovini in serumu smo s citometrično metodo CBA (angl. Cytometric Bead Array) analizirali citokine interlevkin 1β (IL-1β), dejavnik tumorske nekroze α (TNF-α), makrofagni vnetni protein 1α (MIP-1α), makrofagni vnetni protein 1β (MIP-1β), monocitni kemotaktični protein 1 (MCP-1), žilni endotelni rastni dejavnik (VEGF), interlevkin 6 (IL-6), interlevkin 8 (IL-8), interlevkin 10 (IL-10) in interlevkin 12 (IL-12).

Rezultati: Ravni vseh citokinov so se v steklovini pomembno razlikovale od ravni v serumu. Raven MCP-1, VEGF, IL-6, IL-8 v steklovini bolnikov s PDR je bila statistično pomembno višja v primerjavi z ravnijo v steklovini bolnikov v kontrolni skupini, raven MIP-1α, IL-10 in IL-12 v steklovini bolnikov s PDR je bila statistično pomembno nižja v primerjavi z bolniki v kontrolni skupini ($p < 0,05$). Ravni citokinov v serumu bolnikov s PDR se niso pomembno razlikovale od ravni citokinov pri kontrolni skupini. Bolniki z aktivno PDR so imeli v steklovini višjo raven MCP-1, VEGF, IL-6 in IL-8 kot bolniki z inaktivno PDR, a razlike niso bile statistično značilne.

Zaključek: Z našo analizo smo potrdili pomembne razlike v ravneh citokinov med steklovino in serumom pri bolnikih s PDR, kar kaže na pomen lokalnega vnetnega dogajanja v očesu pri PDR. Višje ravni MCP-1, VEGF, IL-6 in IL-8 nakazujejo aktivnost PDR.

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1 Introduction

Diabetic retinopathy (DR) is a late complication of diabetes and remains an important cause of blindness in the working population (1). The first clinically visible changes in the fundus are usually microaneurysms. Damage to the retinal vessels manifests itself as increased vascular permeability and capillary occlusion. Increased vascular permeability leads to retinal edema, and bleeding and hard exudates occur in the retina. As ischemia progresses, neovascularizations begin to form. Depending on the presence of neovascularizations, diabetic retinopathy is divided into two stages: non-proliferative, when neovascularizations are not yet present, and proliferative, when they are already present. Complications of proliferative diabetic retinopathy include vitreous hemorrhage and traction retinal detachment. We also define the condition of the central part of the retina - the macula. If diabetic changes in the macula are present, we speak of diabetic maculopathy or diabetic macular edema. Conditions that seriously compromise vision include complications of proliferative diabetic retinopathy and diabetic macular edema.

The biochemical mechanisms by which hyperglycemia causes tissue changes are not fully elucidated. There are several theories, the most important being the aldose reductase theory, the glycation end product theory, the reactive oxygen species or oxidative stress theory, the protein kinase C theory and the inflammation theory. Although DR is clinically manifested primarily by changes in the retinal vessel, it

can also be defined as chronic subclinical inflammation. Inflammation is reflected by changes at the molecular level, by changes at the cellular level, and by functional changes such as increased vascular permeability and impaired visual function (2).

Cytokines are a diverse group of proteins that are crucial in interactions between inflammatory and other cells. Individual cells can synthesize different cytokines. A single cytokine can affect different cell types, but it can also affect the population of the same cells differently. The effects of individual cytokines are often regulated by other cytokines. The target cells on which the cytokine acts may be the same cytokine-secreting cells, cells in the immediate vicinity or in the same microenvironment, or more distant cells. Cytokines that regulate cell division are called growth factors. Cytokines that affect cell chemotaxis are called chemokines.

Hypoxia and ischemia trigger increased formation of vascular endothelial growth factor (VEGF) in the retina, resulting in increased expression of adhesion molecules and leukostasis. Leukostasis leads to vascular occlusion and therefore additional ischemia, which triggers additional release of inflammatory and angiogenic factors. In addition to VEGF, the synthesis of other inflammatory cytokines and chemokines are increased, such as e.g. tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein (MIP). TNF-α and IL-1β in particular are thought to be crucial for the breakdown of the blood-retinal barrier and for the degeneration of retinal capillaries (3). Angiogenic factors stimulate and modulate the formation of neovascularizations characteristic of proliferative diabetic retinopathy (PDR). In the inflammatory environment, there is a greater activity of matrix metalloproteinases, which degrade the extracellular matrix (4) and thus enable angiogenesis. Matrix metalloproteinase-9 (MMP-9) is induced by IL-8, while the same enzyme also activates IL-8, which recruits more inflammatory cells, creating a destructive positive feedback loop (5).

In several studies, higher levels of VEGF were found in the vitreous of patients with PDR (5-12). VEGF levels correlated with disease activity (10,11,13). Higher levels of MCP-1 (6-8,11,13), IL-6 (7,9) and IL-8 (7,9,12,14) were also found in the vitreous of patients with PDR. The levels of MCP-1, IL-6 and IL-8 in the vitreous of patients with PDR correlated with the level of VEGF (6). Higher levels of IL-6 in the vitreous in patients with PDR correlated with the disease activity (15), and higher levels of IL-8 with ischemia and poorer prognosis of vision outcome (16,17). Some studies have also found higher levels of IL-1β and TNF-α in the vitreous of patients with PDR (9,18) as well as of MIP-1β (19).

Changes in cytokine levels have also been observed in the serum of diabetic patients. In patients with type 1 diabetes as well as in patients with type 2 diabetes, higher levels of C-reactive protein (CRP), IL-6 and TNF-α, and in particular ICAM-1, VCAM-1 and E-selectin, are associated with nephropathy, retinopathy and cardiac vascular diseases (20). Other studies have also found higher levels of TNF-α in the serum of patients with PDR, as well as of VEGF, MCP-1, IL-1β, IL-6 and IL-8 (18,20-22). Some studies did not show differences in the serum levels of the cytokines IL-1β, TNF-α, IL-6, IL-10 and IL-12 in patients with DR compared to the control group (22,23).

With our analysis, we wanted to determine the differences in cytokine levels between vitreous and serum in patients with PDR and in the control group and to compare the levels of cytokines in vitreous with respect to PDR activity. Better knowledge of inflammatory activity in the eye of patients with PDR could contribute to the development of new treatments for these patients.

2 Methods

The study included 37 patients with type 2 diabetes (37 eyes) with PDR that required vitrectomy due to traction deviation, and 20 patients (20 eyes) with idiopathic macular hole (MH) representing the control group.

Exclusion criteria for the group of patients with PDR were: fresh bleeding into the vitreous (less than three months), laser photocoagulation in the last three months, complications during and after surgery, marked changes in the fovea (atrophy, fibrosis, hard exudates), cataracts and glycated hemoglobin (HbA1c) values above 10%. The exclusion criterion for the control group was diabetes. The exclusion criteria for both groups were previous vitrectomy, concomitant presence of another eye disease, systemic inflammatory disease, or haematological disease.

Patients were informed about the purpose and course of the study and joined it voluntarily. Prior to inclusion in the survey, written consent was given for participation. The research was approved by the National Medical Ethics Committee of the Republic of Slovenia (118/12/11). The survey was conducted in accordance with the 1975 Declaration of Helsinki (1983 Revision).

We described the clinical characteristics of patients (age, sex, duration of diabetes, glycated hemoglobin value, body mass index, presence of insulin therapy, arterial hypertension, hyperlipidemia). Data on the patient's general health and diabetes management were obtained from the anamnesis and from the patient's general practitioner or diabetologist. The HbA1c value, expressed as a percentage of glycated hemoglobin, was measured by venous blood analysis one day before surgery. HbA1c reflects the average blood glucose level in the last 6-8 weeks and serves to assess the regulation of diabetes. Arterial hypertension was defined by systolic blood pressure equal to or greater than 140 mm Hg and/or diastolic blood pressure equal to or greater than 85 mm Hg, or based on the information that the patient was taking antihypertensive drugs. Hyperlipidemia was defined on the basis of total cholesterol values greater than 5 mmol/l and/or triglyceride values greater than 2 mmol/l or based on the information that the patient was taking hypolipidemic drugs.

All patients underwent a thorough ophthalmologic examination prior to surgery with determining the best-corrected visual acuity, examination on a biomicroscope, and dilated-pupil fundus examination. We performed gonioscopy and measured intraocular pressure. The fundus examination showed active or inactive neovascularization and obliterated retinal vessels, as well as possible additional changes in the macula. Changes in the ocular fundus were also evaluated during surgery. We took a photo of the patient's fundus with a fundus camera and of the macula with optical coherence tomography (OCT).

Based on the examination of the ocular fundus before surgery and the assessment of the ocular fundus during the procedure, we defined the activity of PDR. The definition of PDR activity was summarized from the study by Aiello et al.: PDR was defined as active retinopathy if blood vessels were visible in the fibrovascular membranes during examination (before and/or during surgery), or as inactive (involutive) retinopathy if only fibrous membranes without blood vessels were present (11).

Just before surgery, a venous blood sample (two tubes) was taken from a vein on the inside of the elbow. At the beginning of vitrectomy, a sample of undiluted vitreous humor (0.5–1.0 ml) was taken. The vitreous humor was absorbed from the central part of the vitreous with a vitreous cutting device into a syringe connected to the vitrectomy system before opening the inflow of balanced salt solution (BSS) through the infusion tube into the eye.

A sample of undiluted vitreous humor taken at the beginning of vitrectomy and

serum were frozen at -80°C and stored until analysis.

Cytokines interleukin 1β (IL-1β), tumor necrosis factor-α (TNF-α), macrophage inflammatory protein-1α (MIP-1α), macrophage inflammatory protein-1β (MIP-1β), monocyte chemoattractant protein-1 (MCP-1), vascular endothelial growth factor (VEGF), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10) and interleukin-12 (IL-12) were analysed in vitreous and control serum using the Cytometric Bead Array (CBA) method.

Statistical analysis. The values of the variables were not normally distributed, so we used the median and the minimum and maximum values for the description. We used the Wilcoxon signed-rank test and the Mann-Whitney test to assess the differences between the compared groups. The Spearman's correlation coefficient was used to estimate the correlation between groups of variables. Due to the large number of tests, we used the Benjamini-Hochberg adjusted P value in the assessment of statistical significance. A value of p less than 0.05 was considered statistically significant. Statistical analysis was performed using the SPSS software, version 19 (SPSS Inc., Illinois).

3 Results

Cytokine levels were determined in vitreous and serum samples from 37 patients with PDR and 20 patients from the control group (MH). The clinical characteristics of the patients are shown in Table 1.

The level of cytokines in the vitreous in patients with PDR was compared with the level of cytokines in the serum (Table 2). The levels of all analysed cytokines in the vitreous were statistically significantly different from the serum levels, and the differences in the levels of MCP-1, VEGF, IL-6, IL-8 stood out the most (p = 0.0025; a higher level in the vitreous than in the serum) and IL-12 (p = 0.0025; a lower level in vitreous than in serum).

Table 1: Clinical features of patients with PDR (n = 37) and the control group with macular hole (MH) (n = 20).

	PDR (n = 37)	FM (n = 20)	p - value
Age (years)	62.5 ± 11.5	71.2 ± 10.2	0.009
Sex	19 men (51.4 %), 18 women (48.6 %)	8 men (40.0 %), 12 women (60.0 %)	0.32
Duration of DB (years)	13.8 ± 8.8	0	
HbA1c (%)	7.9 ± 1.0	0	
Insulin treatment (n)	16 (70.3 %)	0	
Incidence of arterial hypertension (n)	35 (94.6 %)	7 (31.8 %)	0.000
Incidence of hyperlipidemia (n)	21 (56.7 %)	6 (27.3 %)	0.000
BMI	30.7 ± 4.8	24.0 ± 6.3	0.000
NV of iris / angle (n)	2 (5.4 %)	0	
PDR activity	27 active (72.9 %). 10 inactive (27.1 %)	0	

Legend: PDR – patients with proliferative diabetic retinopathy; MH – patients with idiopathic macular hole; DB – diabetes; HbA1c – glycated hemoglobin; BMI – body mass index; NV – neovascularization.

MIP-1α levels were lower than the minimum detectable concentration (<7 pg/ml) in 16 vitreous samples from patients with PDR, as were IL-12 levels in one sample (<1 pg/ml) and IL-10 in two samples (<5 pg/ml). In one serum sample of a patient with PDR, the level of TNF-α was lower than the minimum detectable concentration (<3 pg/ml). In two control group patients, the level of MIP-1β in the vitreous was below the detectable limit of 1 pg/ml.

Comparison of PDR patient samples with MH patient samples is shown in Table 3. Vitreous samples did not differ significantly with respect to IL-1β, TNF-α, and MIP-1β levels, but there were statistically significant differences in other cytokine levels, with the difference in the levels of VEGF, MCP-1, IL-6 and IL-8 standing out the most (p = 0.00025). No significant difference in cytokine levels was found in the serum samples between the groups.

The level of MCP-1 in the vitreous of patients with PDR correlated well with the level of IL-6 in the vitreous (Spearman's coefficient 0.65; p = 0.0001) and with the level of IL-8 (Spearman's coefficient 0.51; p = 0.002). A moderate correlation was also found between VEGF and IL-8 levels (Spearman coefficient 0.43; p = 0.01), between TNFα and IL-10 (Spearman's coefficient 0.42; p = 0.014), between IL-6 and MIP -1β (Spearman's coefficient 0.40; p = 0.02), between MIP-1α and IL-10 (Spearman's coefficient 0.36; p = 0.04), between IL-6 and IL-10 (Spearman's coefficient 0.35; p = 0.04), between IL-12 and IL-10 (Spearman's coefficient 0.35; p = 0.042) and between IL-8 and TNF-α (Spearman's coefficient 0.35; p = 0.045).

The comparison of vitreous samples of patients with PDR (n = 37) with active and inactive form of PDR is shown in Table 4.

Table 2: Comparison of vitreous and serum samples of patients with PDR (median, lowest value - highest value; Wilcoxon signed-rank test).

	Patients with PDR (n = 37)		
	vitreous	serum	p- calue (Benjamini-Hochberg)
IL-1β (pg/ml)	9.15 (7.51–67.36)	7.54 (6.91–15.16)	0.003
TNF-α (pg/ml)	13.4 (2.41–69.09)	5.67 (0–23.35)	0.008
MIP-1β (pg/ml)	12.09 (2.9–38.02)	18.9 (6.89–48.83)	0.006
IL-12 (pg/ml)	3.17 (0–22.26)	17.7 (2.49–42.91)	0.000
MCP-1 (pg/ml)	1404.75 (403.05–3990.74)	72.6 (25.06–227.83)	0.000
MIP-1α (pg/ml)	9.55 (0–82.83)	14.36 (7.78–91.74)	0.001
IL-8 (pg/ml)	93.29 (20.04–295.71)	12.08 (10.12–31.69)	0.000
VEGF (pg/ml)	334.96 (26.41–1439.3)	39.74 (6.17–149.12)	0.000
IL-6 (pg/ml)	60.82 (13.64–798.23)	5.95 (2.41–24.76)	0.000
IL-10 (pg/ml)	5.67 (0–8.12)	7.64 (5.3–15.28)	0.001

Legend: PDR – proliferative diabetic retinopathy; IL-1β –interleukin 1β; TNF-α – tumor necrosis factor α; MIP-1β – macrophage inflammatory protein 1β; IL-12 – interleukin 12; MCP-1– monocyte chemoattractant protein 1; MIP-1α – macrophage inflammatory protein 1α; IL-8 – interleukin 8; VEGF – vascular endothelial growth factor; IL-6 – interleukin 6; IL-10 – interleukin 10.

27 patients with PDR had active disease and 10 patients with PDR had the inactive form of it. Patients with active PDR had higher levels of MCP-1, VEGF, IL-8, and IL-6 in the vitreous, but the differences were not statistically significant.

4 Discussion

Patients with PDR had significantly different levels of all specific cytokines in the vitreous compared to serum. Patients with PDR had significantly different levels of the cytokines IL-12, MCP-1, MIP-1α, IL-8, VEGF, IL-6 and IL-10 in vitreous compared to control group patients. Patients with active PDR had higher levels of MCP-1, VEGF, IL-8, and IL-6 in vitreous compared to patients with inactive PDR, although the differences were not statistically significant. Patients with PDR and the control group had similar serum cytokine levels.

Cytokines play an important role in leukocyte activation, amplification and expansion of local inflammation, and in

Table 3: Comparison of PDR group samples with control group samples with macular hole (MH) (median, lowest value - highest value; Mann-Whitney test).

	Vitreous			Serum		
	PDR (n = 37)	FM (n = 20)	p-value (Benjamini-Hochberg)	PDR (n = 37)	FM (n = 20)	p-value (Benjamini-Hochberg)
IL-1β (pg/ml)	9.15 (7.51–67.36)	10.5 (7.7–35.99)	0.53	7.54 (6.91–15.16)	7.72 (5.55–15.55)	0.89
TNF-α (pg/ml)	13.4 (2.41–69.09)	21.5 (5.16–38.4)	0.29	5.67 (0–23.35)	4.79 (4.0–8.76)	0.79
MIP-1β (pg/ml)	12.09 (2.9–38.02)	13.44 (0–14.4)	0.31	18.9 (6.89–48.83)	17.72 (10.5–27.72)	0.6
IL-12 (pg/ml)	3.17 (0–22.26)	3.99 (2.56–11.18)	0.04	17.7 (2.49–42.91)	11.71 (7.09–18.84)	0.71
MCP-1 (pg/ml)	1404.75 (403.05–3990.74)	374.17 (295.01–881.87)	0.000	72.6 (25.06–227.83)	43.4 (30.24–76.64)	0.1
MIP-1α (pg/ml)	9.55 (0–82.83)	14.25 (9.67–47.34)	0.014	14.36 (7.78–91.74)	20.38 (11.83–91.07)	0.52
IL-8 (pg/ml)	93.29 (20.04–295.71)	14.49 (13.12–16.64)	0.000	12.08 (10.12–31.69)	12.71 (11.21–13.33)	0.8
VEGF (pg/ml)	334.96 (26.41–1439.3)	4.58 (3.5–31.79)	0.000	39.74 (6.17–149.12)	25.73 (17.62–63.61)	0.78
IL-6 (pg/ml)	60.82 (13.64–798.23)	6 (5.28–8.1)	0.000	5.95 (2.41–24.76)	9.14 (5.81–28.4)	0.6
IL-10 (pg/ml)	5.67 (0–8.12)	6.05 (5.84–6.08)	0.04	7.64 (5.3–15.28)	6.63 (5.43–8.43)	0.6

Legend: PDR – group of patients with PDR; MH – group of patients with macular hole; IL-1β – interleukin 1β; TNF-α – tumor necrosis factor α; MIP-1β – macrophage inflammatory protein 1β; IL-12 – interleukin 12; MCP-1 – monocyte chemoattractant protein 1; MIP-1α – macrophage inflammatory protein 1α; IL-8 – interleukin 8; VEGF – vascular endothelial growth factor; IL-6 – interleukin 6; IL-10 – interleukin 10.

modulation of cell proliferation and development of neovascularization. The origin of cytokines in vitreous cannot be precisely determined. They are certainly formed by retinal cells in response to inflammation, but cytokines can also pass into the vitreous from the blood through a broken blood-retinal barrier. They are also formed by inflammatory cells in the vitreous. Regardless of their origin, cytokines affect the development of DR directly and indirectly, and based on the level of cytokines in the vitreous, we can indirectly infer what is happening in the retina.

Higher levels of MCP-1, IL-8, VEGF, IL-6, IL-1β and TNF-α were found in the vitreous of patients with PDR than in the serum.

MCP-1 is the most common chemokine and an important factor in the pathogenesis of inflammatory changes in the retina in DR. It plays an important role in chemotaxis, triggering angiogenesis and fibrosis (24). Higher levels of MCP-1 in the vitreous were found in patients with PDR compared with the control group, and the level in the vitreous was also significantly higher than in serum and correlated with PDR activity (11,19). Our results are consistent with the findings of the aforementioned studies.

Yoshimura et al. found significantly higher levels of IL-6, IL-8, and MCP-1 in the vitreous of patients with PDR and DME and significantly higher levels of VEGF in the vitreous of patients with PDR (6). Several other studies have also found higher levels of IL-6 and IL-8 in the vitreous of patients with PDR (9,17,19). Our results match the results of the mentioned studies. The role of IL-6 and IL-8 in the pathogenesis of PDR is not fully elucidated. IL-6 in vitro can affect the shape of endothelial cells and thus the blood-retinal barrier (25). IL-8, functioning as a chemoattractant, activates neutrophils and T

Table 4: Comparison of vitreous samples of patients with PDR with active and inactive form of PDR (median, lowest value - highest value; Mann-Whitney test).

	PDR (n = 37)		
	active PDR (n = 27)	inactive PDR (n = 10)	p-value (Benjamini-Hochberg)
IL-1β (pg/ml)	8.07 (7.51–67.36)	28.14 (7.71–56.44)	0.41
TNF-α (pg/ml)	13.41 (2.41–69.09)	19.58 (3.51–31.52)	0.64
MIP-1β (pg/ml)	11.85 (2.9–30.67)	16.36 (4.56–38.02)	0.32
IL-12 (pg/ml)	2.66 (0–14.99)	3.80 (1.07–22.26)	0.41
MCP-1 (pg/ml)	1623.19 (437.43–3990.74)	987.23 (403.05–1625.84)	0.06
MIP-1α (pg/ml)	0 (0–68.26)	12.58 (0–82.83)	0.48
IL-8 (pg/ml)	112.76 (37.57–295.71)	54.97 (20.04–275.07)	0.225
VEGF (pg/ml)	477.87 (26.41–1439.30)	188.32 (29.86–1367.13)	0.2
IL-6 (pg/ml)	62.95 (20.41–798.23)	32.82 (13.64–220.31)	0.42
IL-10 (pg/ml)	5.57 (0–6.9)	5.79 (5.37–8.12)	0.26

Legend: PDR – vitreous samples from patients with PDR; IL-1β – interleukin 1β; TNF-α - tumor necrosis factor α; MIP-1β – macrophage inflammatory protein 1β; IL-12 – interleukin 12; MCP-1 – monocyte chemoattractant protein 1; MIP-1α – macrophage inflammatory protein 1α; IL-8 – interleukin 8; VEGF – vascular endothelial growth factor; IL-6 – interleukin 6; IL-10 – interleukin 10.

lymphocytes, and is also angiogenic. Its level in vitreous was found to correlate with PDR activity (11). The breakdown of the blood-retinal barrier alone is probably not the only and sufficient reason for higher levels of IL-6 and IL-8 in the vitreous. Both cytokines are more likely to be also formed by cells present in the vitreous of patients with PDR (macrophages, monocytes, glial cells, retinal pigment epithelial cells).

VEGF is a proangiogenic cytokine that is crucial for the development of neovascularizations and also has a significant effect on the blood-retinal barrier. Numerous studies have demonstrated higher levels of VEGF in patients with PDR (6,8-11) and the correlation of VEGF with disease activity (5,10,11,13). In our study also, we found higher levels of VEGF in the vitreous of patients with PDR both compared to serum and compared to the vitreous of the control group. Patients with active PDR had higher levels of VEGF in vitreous than patients with inactive PDR, but the differences were not statistically significant.

IL-1β is a pro-inflammatory cytokine that has been shown to play an important role in the development of DR in animal model studies. Because it has a short half-life and is formed predominantly in the tissue, only a small number of studies have demonstrated a higher level in the vitreous of diabetic patients compared with the control group (18,26). In a study by Yoshimura et al., IL-1β in the vitreous of patients with PDR was not proven because concentrations were below the detectable limit of the method used (6). In our study, the level of IL-1β in vitreous did not differ significantly between the group of patients with PDR and the control group.

TNF-α is a pro-inflammatory cytokine involved in the pathogenesis of many chronic inflammatory diseases. In patients with diabetes, a higher TNF-α level in serum was observed compared to the control group and a strong correlation between TNF-α levels in serum and DR progression (18,21). In a study conducted by Yoshimura et al. (6), the level of TNF-α in the vitreous of patients with PDR was undetectable, and in our study it was higher in the vitreous of patients with PDR than in the serum, but did not differ significantly from the level in the vitreous of patients in the control group. TNF-α also has a short half-life similar to IL-1β, which may give false negative results.

In our study, levels of IL-12, IL-10 as well as MIP-1α and MIP-1β were significantly lower in vitreous than in serum.

IL-12 is a pro-inflammatory cytokine with antiangiogenic activity (27). There are very few studies that have looked at IL-12 in people with diabetes. Children with type 1 diabetes and DR were found to have significantly lower IL-12 levels in serum compared with the control group (27). Our study found a significantly lower level of IL-12 in the vitreous of patients with PDR compared with the serum. The level of IL-12 in the vitreous of patients with PDR was also significantly lower compared to the control group, although the values varied considerably.

Little is known about the possible role of anti-inflammatory cytokines in the pathogenesis of PDR. IL-10 is an anti-inflammatory cytokine that stops macrophages and inhibits VEGF expression. Mao et el. found higher levels of IL-10 in the vitreous of patients with PDR (28), which is consistent with the protective role of IL-10 in the inflammatory environment. On the contrary, Hernandez et al. did not find higher levels of IL-10 in the vitreous of patients with PDR, suggesting that higher levels of pro-inflammatory cytokines did not cause an increase in cytokine levels with the opposite effect (29). Similarly, Zhou et al. did not find a significant difference between the level of IL-10 in the vitreous of patients with PDR and the level of IL-10 in the vitreous of the control group (10). Our results are in line with the conclusions of Hernandez et al. and Zhou et al., as the level of IL-10 in the vitreous of our patients with PDR was lower than the level

in the vitreous of the control group.

MIP-1α and MIP-1β are formed by macrophages, act chemotactically, and are also important in the pathogenesis of retinal neovascularization (30). Polymorphonuclear cells activated by MIP-1α and MIP-1β release cytokines such as IL-1β, IL-6 and TNF-α. In a study by Maier et al., no levels of MIP-1α and MIP-1β were detected in vitreous samples neither in PDR patient group nor in the control group, and Capeans et al. as well did not prove the presence of MIP-1α and MIP-1β in vitreous samples of the PDR patient group nor the control group (14,30). Capeans et al. based on the results of her study, concluded that MIP-1α and MIP-1β are unlikely to be involved in the pathogenesis of PDR. Yoshimura et al. proved the presence of MIP-1β only in the vitreous of some patients with PDR, but the level of MIP-1α was lower than the minimum detectable concentration (6). In our study, MIP-1α levels were above the minimum detectable limit in 21 of 37 vitreous samples from patients with PDR (56.7%) and in all control group vitreous samples (100%), the differences in levels were statistically significantly different, and variability was very high in the group of patients with PDR. The level of MIP-1β was lower than the minimum detectable concentration in only two vitreous samples of the control group, and the presence of MIP-1β was proven in all vitreous samples of patients with PDR. The differences between the groups were not statistically significant. There was no significant difference in MIP-1α and MIP-1β levels between the group of patients with active PDR and the group of patients with inactive PDR. Based on our results, we can confirm the conclusion of Capeans et al. that MIP-1α and MIP-1β probably do not play a significant role in the pathogenesis of DR. A statistically significant difference between the group of patients with PDR and the control group, which was determined according to the level of MIP-1α in the vitreous, can be attributed to the greater variability of levels in individual samples (ranging 0–82.83 pg/ml in patients with PDR compared to the range of 9.67–47.34 pg/ml in the control group). This may also indirectly indicate the likely minor importance of MIP-1α in the pathogenesis of DR.

5 Conclusion

Inflammation in the eye is associated with changes that occur as part of diabetic retinopathy. Our study demonstrated significant differences in cytokine levels between vitreous and serum in patients with PDR. Higher levels of MCP-1, VEGF, IL-6 and IL-8 and lower levels of MIP-1α, IL-10 and IL-12 in the vitreous of patients with PDR compared to the control group further confirm the importance of local inflammation in the eye in diabetic retinopathy. Higher levels of MCP-1, VEGF, IL-6 and IL-8 in the vitreous indicate PDR activity. Better knowledge of pathogenetic mechanisms and local inflammatory activities in the eye can contribute to the development of more effective treatment methods.

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