Original Article

Proliferative and inflammatory factors in the vitreous of patients with proliferative diabetic retinopathy

VV Chernykh[1], EV Varvarinsky[1], EV Smirnov[1], DV Chernykh[1], Alexander N Trunov[1,2]

Purpose: The purpose was to measure the concentrations of various cytokines and growth factors (including vascular endothelial growth factor [VEGF] and pigment epithelium-derived factor [PEDF]) in the vitreous of patients with proliferative diabetic retinopathy (PDR) and to investigate interaction between inflammatory and proliferative factors in the genesis of PDR. **Materials and Methods**: Vitreous samples from 32 eyes with PDR and 25 eyes without diabetes mellitus and signs of DR (control) were collected. Vitreous concentrations of VEGF, PEDF, monocyte chemotactic protein-1 (MCP-1), interleukin-4 (IL-4), IL-6, IL-8, IL-10, IL-17A, and secretory immunoglobulin A (sIgA) were simultaneously measured using enzyme-linked immunoassay. **Results**: Vitreous levels of VEGF, PEDF, IL-17A, IL-6, IL-8, IL-4, and sIgA were significantly ($P < 0.05$) higher in eyes with PDR compared to control. The concentration of VEGF was more than 17-times higher than in control, and the concentration of PEDF was not changed oppositely and was also higher (1.45-times) compared to control, that may indicate disturbances of compensatory mechanisms in angiogenesis regulation in PDR. Significant ($P < 0.05$) positive correlations were observed between vitreous concentrations of VEGF and IL-17A ($r = 0.45$), VEGF and IL-8 ($r = 0.48$), VEGF and IL-4 ($r = 0.51$), PEDF and IL-17A ($r = 0.48$), PEDF and IL-8 ($r = 0.59$), MCP-1 and PEDF ($r = 0.72$), MCP-1 and IL-8 ($r = 0.45$), IL-4 and IL-17A ($r = 0.65$), IL-4 and IL-8 ($r = 0.71$), IL-8 and IL-17A ($r = 0.59$). **Conclusions:** Significantly raised levels of inflammatory and proliferative factors and numerous positive correlations between them may demonstrate a significant role of activation of vascular proliferation and local inflammation in the pathogenesis of PDR.

Key words: Cytokines, diabetic retinopathy, pigment epithelium-derived factor, vascular endothelial growth factor

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Diabetic retinopathy (DR) remains one of the most severe ocular complications of diabetes and a major cause of vision loss in many countries. The development and progression of DR depend on duration of diabetes, efficacy of multidisciplinary therapeutic interventions and other factors. Progression of proliferative DR can be complicated by recurrent vitreous hemorrhages, traction retinal detachment, retinal and vitreous fibrosis, optic nerve atrophy, which leads to severe visual impairment.[1-6]

Research in recent years has emphasized the role of the vascular endothelial growth factor (VEGF) and other bioactive substances with angiogenic and antiangiogenic activity (such as the pigment epithelium-derived factor [PEDF]) in the pathogenesis of proliferative DR (PDR).[6-10]

A number of studies of PDR pathogenesis have demonstrated disturbances reflecting activation of inflammation, functional imbalance in the immune system (including cytokines and matrix metalloproteinase imbalance), as well as activation of vascular proliferation.[11-16]

[1]S. N. Fyodorov Federal State Institution, Intersectoral Research and Technology Complex, Eye Microsurgery, Ministry of Health of Russian Federation, Novosibirsk Branch, Vitreoretinal Department, [2]Research Centre of Clinical and Experimental Medicine, Siberian Branch of the Russian Academy of Medical Sciences, Immunological Department, Novosibirsk, Russia

Correspondence to: Prof. Alexander N Trunov, 10 Kolhidskaya Street, Novosibirsk 630071, Russia. E-mail: trunov1963@yandex.ru

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Although recent studies have elucidated different aspects of mechanisms of PDR onset and progression, many issues related to interactions of pro-/antiinflammatory and regulatory cytokines network and angiogenic factors remain disputable.

Therefore, the purpose of the current study was to investigate vitreous concentrations of a number of pro-/anti-inflammatory and angiogenic factors and their interactions in activation of inflammation and proliferation in PDR.

Materials and Methods

Subjects

Sixty-three patients (63 eyes) with tractional retinal detachment who underwent vitrectomy were examined and recruited in 2011–2012. The study was conducted in accordance with the declaration of Helsinki and approved by the Ethics Committee of the Aforementioned Institution. All patients signed an informed consent form prior to participation in the study (noting that they agree to receive a surgical treatment, give a sample of the vitreous and are aware that the study results will be used for scientific purposes).

Patients were divided into two groups based on the presence or absence of PDR. The study group comprised of 38 patients (38 eyes) with PDR and tractional retinal detachment (inclusion criteria = presence of diabetes and PDR). The control group consisted of 25 patients (25 eyes) with tractional retinal detachment and with no history of diabetes and DR (inclusion criteria = no diabetes and PDR). An exclusion criterion for participation in this study was clinical evidence of acute or exacerbation of chronic inflammatory

ocular diseases (uveitis of any etiology), primary open-angle glaucoma, systemic autoimmune disorders, and tumors of any localization.

Assuming that subjects in the study and control groups had a similar level of retinal damage due to retinal detachment, the differences in vitreous concentrations of studied factors in the respective groups are likely to be attributable to the presence or absence of DR.

Diagnosis of PDR was made based on visual acuity measurement, visual field assessment, binocular ophthalmoscopy with ophthalmoscope (Heine Omega 200) and 20D lens, slit lamp examination (Karl Zeiss SL 115 Classic) and 78D lens (Ocular Max Field), and ultrasound (Tomey UD 1000).

Preoperatively and postoperatively, patients in both study groups received standard therapy as routinely prescribed at Novosibirsk branch of the academician S. N. Fyodorov Eye Microsurgery Federal State Institution. It included conjunctival sac instillations of 0.1% diclofenac sodium (Naclof eye drops), 0.1% dexamethasone eye drops-one drop 3-times a day for 10 days; 0.3% tobramycin (Tobrex eye drops)-one drop 2-times a day for 14 days, 1% tropicamide eye drops-one drop 3-times a day for 7 days.

Vitreous sample collection

The standard three-port 23G, 25G posterior vitrectomy was performed in both study groups using the Constellation Vision System (Alcon, USA), Stellaris PC (Bausch and Lomb, USA) and Assistant (Opticon, Italy). The vitreous samples collected at the initial stage of vitrectomy were used as the study material. To ensure that the balanced salt solution did not mix up to the vitreous sample, the latter was collected at the stage of the air tamponade. This was followed by reattachment of the retina, retinal tamponade with perfluoro-n-octane and endolaser coagulation.

The obtained samples were placed in a tube and centrifuged at 1500 rpm for 10 min. Then the top layer was collected, placed into sterile plastic tubes and frozen at −70°C. The samples were assayed within 6 months after collection.

Vitreous sample analyses

All the laboratory analyses of vitreous samples were performed using commercially available enzyme immunoassay, according to the instructions in the manuals. VEGF, monocyte chemoattractant protein-1 (MCP-1) and secretory immunoglobulin A (sIgA) were measured using "Vector-Best" kit (Russia). PEDF was measured using "CUSABIO" kit (China). Interleukins (IL) 4, 6, 8, 10, and 17A were measured using "Cytokine" kit (Russia).

Results of the enzyme immunoassay were registered using vertical photometer "Uniplan" (Russia) at 450 nm wavelength.

Statistical analysis

All statistical analyses were performed using STATISTICA, version 10 software package (StatSoft Inc., USA). To investigate differences in variables between the two study groups we used the Mann–Whitney independent samples *t*-test. Numbers were expressed as mean ± standard error of the mean. Correlation between vitreous concentrations of cytokines was analyzed using Spearman's rank correlation test. A $P < 0.05$ was considered significant (95% confidence interval).

Results

Mean age of the study population was 52.6 ± 4.5 years, 29 were males and 34 were females. Mean age in the study group was 50.5 ± 3.2 years (16 males and 22 females) and in the control group 53.5 ± 2.6 years (13 males and 12 females). In the study group, only 8 patients had diabetes mellitus (DM) type I and 30 patients had DM type II. For all patients with diabetes, its duration was more than 8 years. Table 1 presents the data obtained from analysis of the vitreous samples.

The concentrations of VEGF, a factor which promotes angiogenesis, in the vitreous of patients in the study group were more than 17-times higher than in controls, and this difference was highly significant ($P < 0.001$). Vitreous levels of PEDF were significantly, 1.45-times higher than in the control group ($P < 0.05$).

Concentrations of the pro-inflammatory cytokine IL-17A in the vitreous of patients and PDR were significantly, 4.5-times higher than in the patients with tractional retinal detachment without PDR ($P < 0.01$).

Vitreous levels of both IL-6 and IL-8 were 1.9-times higher in study group than in the control patients ($P < 0.01$).

Concentrations of MCP-1 in the vitreous of patients from the study group were significantly, 2.2-times higher than in the patients from the control group ($P < 0.01$).

Concentrations of the anti-inflammatory cytokine IL-4 in the vitreous body of patients from the study group were significantly, 4-times higher, then in the control group ($P < 0.01$).

Vitreous IL-10 concentrations were not significantly differ between the two groups.

Vitreous levels of sIgA were also significantly higher compared to the control group ($P < 0.01$).

Results obtained in the correlation analysis are presented in Table 2.

Discussion

Vascular endothelial growth-factor concentrations have been shown to be significantly elevated in the study group. These

Table 1: Vitreous levels of studied factors

Variables	Control group (*n*=25)	Study group (*n*=38)
VEGF, pg/ml	88.8±27.7	1520.1±178.3*
PEDF, pg/ml	190.4±8.3	279.1±42.7*
IL-17A, pg/ml	676±4.6	311.2±98.2*
IL-4, pg/ml	8.1±0.5	32.5±9.4*
IL-6, pg/ml	32.8±8.7	64.2±14.6*
IL-8, pg/ml	28.7±1.23	55.4±16.7*
IL-10, pg/ml	4.47±0.24	4.43±0.69
MCP-1, pg/ml	447.1±35.8	990.6±108.6
sIgA, mg/L	0.96±0.05	1.43±0.13*

The values are presented as mean±SEM. *Differences between groups are significant at the $P<0.05$ level. SEM: Standard error of mean, VEGF: Vascular endothelial growth factor, PEDF: Pigment epithelium-derived factor, MCP-1: Monocyte chemotactic protein-1, IL-4: Interleukin-4, sIgA: Secretory immunoglobulin A

Table 2: Correlations between the studied variables in the vitreous of patients with proliferative diabetic retinopathy

Indicator 1	Indicator 2	Correlation coefficient (*r*)	*P*
VEGF	IL-17A	0.45	<0.05
VEGF	IL-8	0.48	<0.05
PEDF	IL-17A	0.48	<0.05
PEDF	IL-8	0.59	<0.04
MCP-1	PEDF	0.72	<0.01
MCP-1	IL-8	0.45	<0.05
IL-4	IL-17A	0.65	<0.02
IL-4	IL-8	0.71	<0.01
IL-4	VEGF	0.51	<0.05
IL-17A	IL-8	0.59	<0.04

VEGF: Vascular endothelial growth factor, PEDF: Pigment epithelium-derived factor, MCP-1: Monocyte chemotactic protein-1, IL-4: Interleukin-4

findings are in agreement with the previous reports on VEGF levels in patients with PDR.[7,8,10,17]

Pigment epithelium-derived factor in intraocular angiogenesis is regarded as strong antiangiogenic factor that suppresses the formation of new blood vessels trough inhibiting migration and proliferation of endothelial cells. Significantly elevated concentrations of PEDF in the present study contradicts the findings of a number of studies that have shown the increase in VEGF and associated decrease in PEDF in the vitreous of patients with PDR.[8,18,19] These contradictory results can potentially be explained by the difference in the duration of DR, and the increase in PEDF can be considered a compensatory mechanism aimed to reduce angiogenesis. However, this requires further investigation.

It has been previously reported that the IL-17A produced by T cells stimulates synthesis of a number of pro-inflammatory cytokines (tumor necrosis factor-α, IL-1β), intercellular adhesion molecules and other biologically active substances.[12,20] Therefore, the increase in the IL-17A in patients with PDR emphasizes the role of the local inflammatory process in the pathogenesis of PDR, also suggested by the similar results obtained for a number of other pro-inflammatory cytokines.

Interleukin-6 is a pro-inflammatory cytokine contributing to the transformation from acute to chronic inflammation and development of autoimmune reactions.[21] The levels of IL-8 as a factor responsible for inflammatory-destructive processes changed similarly to IL-6 levels and were significantly higher in the vitreous of patients with PDR. These results are in agreement with the previous reports on concentrations of studied cytokines in patients with PDR.[10,15,17] The simultaneous increase of both IL-6 and -8 concentrations may reflect the significance of inflammation-induced vascular damage in DR pathogenesis.

Angiogenic activity of MCP-1, as reported in experimental studies, is comparable to the VEGF activity. MCP-1 has been shown to stimulate intensive macrophage recruitment and induce the chemotaxis of human endothelial cells promoting pathologic neovascularization.[22,23] Based our findings indicating the potential role of MCP-1 in intraocular angiogenesis, it is reasonable to hypothesize that VEGF is not the only factor involved in angiogenesis activation in PDR, which is also suggested by the previous reports published on the increase of MCP-1 in PDR and the opinion of the authors on the role of this protein in angiogenesis activation when inflammation is involved.[17,24]

When analyzing concentrations of IL-10, an immunosuppressive cytokine contributing to regulation of VEGF synthesis, we did not reveal any significant increase compared in patients with DR. However, other studies have reported raised concentrations of IL-10 in PDR and it is considered to be important in the pathogenesis of this disease. The result obtained in our study can potentially be attributed to exhaustion of compensatory reactions.[15]

This rise of the anti-inflammatory cytokine IL-4 can be interpreted not only as a compensatory reaction aiming to reduce inflammation associated with the PDR, but also as a sign of the humoral immune response, and this speculation is supported by the significantly increased concentrations of sIgA.

Correlation analysis revealed numerous significant positive correlations between vitreous concentrations of studied factors that may indicate the association of local inflammation and vascular proliferation activity in the pathogenesis of PDR.

Conclusions

The current study has shown that the local inflammatory-immune process plays an important role in the mechanisms of vascular damage in PDR, as evidenced by significantly higher levels of pro-inflammatory cytokines IL-17A, IL-8, IL-6, MCP-1, humoral immune system inductor IL-4 and sIgA, in the vitreous of patients from the study group when compared to controls. The aforementioned processes are associated with activated vascular proliferation and manifest in more than 17-times increased concentrations of VEGF in the vitreous of patients in the study group. The study has also shown that there are probably numerous mechanisms involved in activation of the vascular proliferation, one of which is related to increased production of MCP-1. The correlations observed also indicate that there is a link between the inflammatory processes and vascular proliferation in the pathogenesis of PDR. Further understanding of the role of angiogenic and inflammatory factors may provide new insights into targeting and prevention of PDR.

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