• Clinical Research •

Optimal timing of preoperative intravitreal anti-VEGF injection for proliferative diabetic retinopathy patients

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Abstract

● **AIM:** To analyze concentrations of vascular endothelial growth factor (VEGF) and fibrosis-related factors in vitreous fluid of proliferative diabetic retinopathy (PDR) patients pre-treated with intravitreal anti-VEGF injections (IVI) at different time periods prior to pars plana vitrectomy (PPV), and their correlation with the degree of vitreoretinal fibrosis and explore the optimal timing of preoperative IVI.

● **METHODS:** The prospective case-control study from January 2019 to July 2020 included 31 eyes with PDR-related complications (PDR group) and 21 eyes with non-diabetic ocular disease (control group) requiring PPV. PDR eyes were divided into four groups based on timing of PPV: 3d after IVI (3-day group); 5d after IVI (5-day group); 7 or more days after IVI (≥7-day group); and no IVI. Vitreous fluid samples (0.5-1.0 mL) were collected prior to switching on the infusion before routine 23-G PPV. Concentrations of VEGF, basic fibroblast growth factor (bFGF), periostin (PN), interleukin (IL)-6, IL-8, and tumor necrosis factor (TNF)-α were measured by immunoassay, and concentration differences for each cytokine were compared among the groups. The degree of vitreoretinal fibrosis was graded intraoperatively, and the correlation between the changes in cytokine levels and the severity of vitreoretinal fibrosis was analyzed by univariate ordinal logistic regression analysis.

● **RESULTS:** PDR eyes without IVI had significantly higher VEGF, bFGF, PN, and IL-6 concentrations than non-diabetic eyes (all $P<0.05$), and had a significantly higher concentration of VEGF ($P<0.05$) and a significantly lower concentration of IL-8 ($P<0.05$) than PDR eyes with IVI. Statistically significant differences were also observed for concentrations of VEGF, bFGF, PN, IL-6, and IL-8 among 3-day, 5-day, and ≥7-day groups (all $P<0.05$); meanwhile there was no significant difference in TNF-α among groups ($P=0.226$). The 5-day group had the lowest concentration of VEGF and the ≥7-day group had the highest concentration of bFGF and PN. The degree of vitreoretinal fibrosis was significantly higher in the ≥7-day group compared to the 3-day ($P=0.015$) and 5-day group ($P=0.039$), and vitreoretinal fibrosis correlated significantly with concentrations of bFGF, PN, IL-6, and IL-8 (all $P<0.05$). Univariate ordinal logistic regression analysis showed that bFGF was an independent risk factor for the severity of vitreoretinal fibrosis in PDR patients pre-treated with IVI.

● **CONCLUSION:** The vitreous concentrations of VEGF, bFGF, PN, IL-6, and IL-8 change after pretreatment with IVI before PPV in PDR patients. The degree of vitreoretinal fibrosis is higher in patients with a longer time between IVI treatment and PPV, which may be related to the angio-fibrosis switch. The results suggest that PPV should be performed 5d after IVI administration in PDR patients.

● **KEYWORDS:** preoperative timing; intravitreal anti-VEGF injection; angio-fibrosis switch; proliferative diabetic retinopathy

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INTRODUCTION

Proliferative diabetic retinopathy (PDR) is a serious ocular microvascular complication of diabetes characterized by pathological neovascularization with or without fibrovascular proliferation[1-3]. Disease progression leads to contraction of proliferative fibrovascular membranes (FVMs) that can cause vitreous hemorrhage (VH) and tractional retinal detachment (TRD) eventually leading to blindness. Many studies have

confirmed that vascular endothelial growth factor (VEGF) is an important inducing factor involved in this pathological process, and its levels are closely related to the severity of PDR[4-7]. Therefore, in the past 10y, VEGF inhibitors (anti-VEGFs) have been widely used as adjunct treatment for PDR patients. At present, intravitreal anti-VEGF injections (IVI) are performed as an important ancillary treatment in combination with pars plana vitrectomy (PPV) in PDR patients[8-9].

It is undeniable that the clinical results of PDR patients treated with preoperative IVI combined with PPV are significantly better than those of patients treated with PPV alone. Numerous retrospective studies have confirmed that preoperative IVI can promote regression of neovascularization, minimize intraoperative bleeding during segmentation of FVMs, shorten operation time, and reduce the occurrence of postoperative VH, as well as decrease the likelihood of other intraoperative and postoperative-associated complications[10-13]. Moreover, the visual prognosis of patients treated with preoperative IVI combined with PPV was better than that of patients treated with PPV alone.

Although the emergence of anti-VEGF drugs marks a great progress in the treatment of PDR, an increasing number of studies have provided evidence that IVI is effective in some PDR patients, while others develop progressive severity of fibrosis, especially when the interval between IVI and PPV is long[14-17]. Li *et al*[18] showed that the timing of anti-VEGF therapy played an important role in the development of fibrosis, supporting the idea that the timing of preoperative IVI can be an important factor in determining whether anti-VEGF treatment will be a beneficial or detrimental adjunct to PPV.

At present, the mechanism of fibrosis aggravation after IVI remains unclear. Klaassen *et al*[19] proposed that this phenomenon was related to the angio-fibrotic switch. In other words, at some point there was a transition from angiogenesis inhibition to fibrosis promotion. Therefore, we theorize that IVI not only blocks VEGF but also causes changes in the concentrations of other cytokines in PDR patients.

The aim of the present study was to analyze the concentrations of various cytokines in the vitreous fluid and to correlate this with the severity of fibrosis in PDR patients based on different lengths of time between IVI and PPV, and to elucidate the specific molecular mechanism of the angio-fibrosis switch, so as to provide a reference for retinal surgeons to the optimal timing for anti-VEGF treatment.

SUBJECTS AND METHODS

Ethical Approval The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the institutional review board and Ethical Committee of the First Affiliated Hospital of Anhui University of Science & Technology. All patients voluntarily signed informed consent after having been provided with a detailed description of the treatment.

Patients We conducted a prospective case-control study of 31 eyes of 31 PDR patients (PDR group) and 21 eyes of 21 non-diabetic controls including patients with macular hole or rhegmatogenous retinal detachment (control group) who underwent 23-G PPV at the Ophthalmology Department of the First Affiliated Hospital of Anhui University of Science & Technology in Huainan, China, between January 2019 and July 2020.

The surgical indications of the PDR group included persistent VH and TRD with proliferative FVMs. The exclusion criteria included the following: 1) a history of previous ocular surgery (*e.g.*, phacoemulsification, trabeculectomy), 2) previous treatment with anti-VEGF therapy or pan-retinal photocoagulation within 6mo, 3) a history of topical anti-inflammatory drops like as nepafenac, 4) presence of other retinal neovascular diseases (*e.g.*, retinal vein occlusion), 5) presence of other ocular diseases that seriously affect visual function (*e.g.*, keratitis, optic neuritis, uveitis, high myopia, and amblyopia).

Eligible PDR patients were divided into two groups, one group was pre-treated with IVI prior to PPV (n=26; IVI PDR group) and the other group was not pretreated with IVI (n=5; no IVI group). The IVI PDR group was further subdivided into three groups based on the timing of pretreatment: 1) PDR patients who underwent PPV 3d after IVI (n=9; 3-day group); 2) PDR patients who underwent PPV 5d after IVI (n=10; 5-day group); 3) PDR patients who underwent PPV ≥7d after IVI (n=7; ≥7-day group). Two kinds of anti-VEGF drugs were used in IVI PDR group, of which 3, 6, and 3 eyes were pre-treated with intravitreal conbercept injections respectively in 3-day group, 5-day group and ≥7-day group and 6, 4, 4 eyes were pre-treated with ranibizumab respectively. There was no statistical difference in the types of anti-VEGF drugs among subgroups (χ^2=1.397, P=0.497).

Examinations All patients underwent comprehensive preoperative ophthalmic and medical examinations. Eye examinations included slit-lamp biomicroscopy, intraocular pressure (IOP) measurement, ocular B-scan ultrasonography, ultra-wide-angle fundus photography, and spectral domain optical coherence tomography (SD-OCT). Fundus fluorescein angiography (FFA) was used to assess macular perfusion status if necessary.

The degree of vitreoretinal fibrosis in PDR patients was graded according to the classification reported by Kuiper *et al*[20] as follows: grade 0, no fibrosis; grade 1, few pre-retinal membranes; grade 2, white preretinal fibrotic membranes with limited extension into the vitreous; grade 3, abundant white membranes reaching into the vitreous body.

Sample Collection Undiluted vitreous fluid samples (0.5-1 mL) were obtained before PPV without switching on the infusion fluid. The samples were centrifuged at 3000 g for 10min at 4℃ and the supernatant was immediately transferred to a different sterile Eppendorf® tube. All preliminary centrifuged samples were stored in the refrigerator at -80℃ until further analysis.

Measurement of Cytokines *via* Luminex Technology A human premixed multi-analyte kit (R&D Systems, Austin, TX, USA) was used to measure the concentrations of cytokines in vitreous fluid samples. The cytokines measured included VEGF, basic fibroblast growth factor (bFGF), periostin (PN), interleukin (IL)-6, IL-8, and tumor necrosis factor (TNF)-α. The preliminary centrifuged samples were diluted 2-fold with calibrator diluent RD6-52. First, 50 μL of the diluted sample and microparticle cocktail were added to each well of the microplate and incubated for 2h at room temperature on a horizontal orbital microplate shaker (0.12s orbit) set at 800±50 rpm. Afterwards, the microplate was washed by removing the liquid from each well, filling the wells with 100 μL wash buffer, and removing the liquid again; this procedure was performed three times. Next, 50 μL of diluted biotin-antibody cocktail were added to each well and incubated for 1h at room temperature on the shaker set at 800 rpm. Subsequently, the wash was repeated as described above, followed by the addition of 50 μL of diluted Streptavidin-PE to each well and incubation for 30min at room temperature on the shaker set at 800 rpm. Following this, the wash was repeated for the third time and 100 μL of wash buffer were added to each well and incubated for 2min at room temperature on the shaker set at 800 rpm. Finally, the concentration values were obtained from the mean fluorescent intensity (MFI) by using Luminex 200™ System (xPONENT Software). Standard curves were generated from the reference cytokine gradient concentrations. The concentrations of cytokines in vitreous fluid samples were calculated from the standard curves.

Statistical Analysis SPSS 22.0 software (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. The Shapiro-Wilk test was performed to examine whether the data were distributed normally. Data are presented as the mean±standard deviation (SD) or as median and range. Statistical significances of cytokine concentration among the groups were determined using the Kruskal-Wallis *H* test with Bonferroni post hoc test, while differences between two groups were estimated using a non-parametric Mann-Whitney *U* test. A χ^2 or Fisher exact test was conducted to compare noncontinuous variables. The spearman rank correlation test was used to analyze the correlation between the degree of vitreoretinal fibrosis and the concentration of cytokines. Based on the results of univariate analysis, the degree of vitreoretinal fibrosis was taken as the dependent variable, and the factors of $P<0.05$ were included in the multivariate regression model for multivariate ordered logistic regression analysis. A *P*-value <0.05 was considered statistically significant.

RESULTS

Patient Characteristics Characteristics of the 52 patients studied are shown in Table 1. Statistical analysis demonstrated that there was no significant difference in sex ($P=1.000$) and age ($P=0.178$) among the IVI PDR group, no IVI PDR group, and the control group. There was also no significant difference in hemoglobin A1c (HbA1c) and indication for PPV between the pretreated and non-pretreated PDR groups ($P=0.755$, 0.525, 1.000, and 1.000, respectively; all $P>0.05$; Table 1).

Vitreous Fluid Cytokine Concentrations The cytokine concentration values for the IVI PDR group, no IVI PDR group, and the control group are shown in Table 2. The concentrations of VEGF, bFGF, PN, and IL-6 were all significantly higher in the no IVI group than in the control group ($Z=-3.420$, -2.279, -2.950, and -3.027, respectively; all $P<0.05$), while no significant differences were observed in IL-8 and TNF-α between the two groups (both $P>0.05$). In addition, the IVI group had a significantly lower concentration of VEGF ($Z=-3.491$, $P<0.05$) and a significantly higher concentration of IL-8 ($Z=-2.363$, $P<0.05$) than the no IVI group. While there were no significant differences in bFGF, PN, IL-6, and TNF- α concentrations between the two groups (all $P>0.05$).

There were significant differences in VEGF, bFGF, PN, IL-6, IL-8, and TNF-α concentrations in the vitreous fluid among the five groups ($F=42.105$, 36.653, 33.396, 28.517, 26.554, and 18.326, respectively; all $P<0.01$; Figure 1).

We further compared the concentrations of cytokines among the 3-day group, 5-day group, and ≥7-day group and discovered that the differences in VEGF, bFGF, PN, IL-6, and IL-8 concentrations were statistically significant ($F=13.077$, 16.524, 10.440, 7.748, and 8.960, respectively; all $P<0.05$). There was no significant difference among the 3 subgroups in TNF-α concentration ($F=2.978$, $P=0.226$).

Vitreous fluid samples in the 5-day group had the lowest concentration of VEGF, and this was statistically significant compared with the 3-day group ($P=0.002$) and the ≥7-day group ($P=0.024$). However, there was no significant difference in the concentrations of vitreous VEGF between the 3-day group and ≥7-day group ($P>0.05$).

In addition, vitreous fluid samples in the ≥7-day group had the highest concentrations of bFGF and PN, and this was statistically significant compared with the 3-day group ($P=0.000$, $P=0.008$, respectively) and the 5-day group ($P=0.012$, $P=0.020$, respectively). No significant differences were found in the concentrations of vitreous bFGF and PN between the 3-day group and 5-day group (both $P>0.05$).

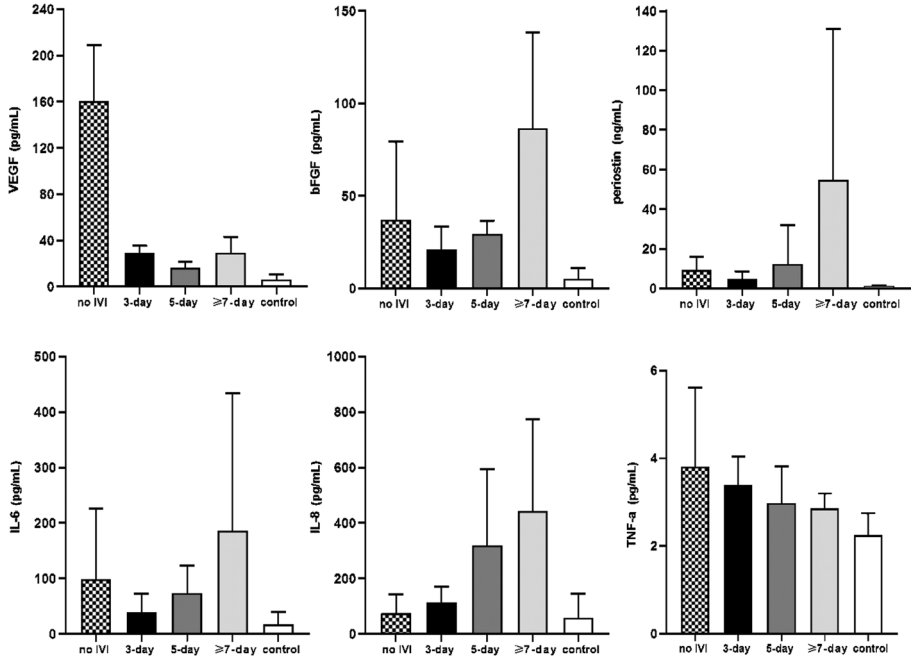

Figure 1 Concentrations of cytokines in vitreous fluid of patients in each group VEGF: Vascular endothelial growth factor; IVI: Intravitreal anti-VEGF injections; bFGF: Basic fibroblast growth factor; IL: Interleukin; TNF: Tumor necrosis factor.

Table 1 Patient characteristics

Characteristics	PDR group		Control group	*P*
	No IVI group	IVI group		
Vitreous sample, *n*	5	26	21	
Sex, male/female	3/2	13/13	10/11	1.000[a]
Age, y, mean±SD	61.40±8.56	55.92±10.37	61.24±10.28	0.178[b]
Hemoglobin A1c, %, mean±SD	7.68±1.87	7.89±1.25	-	0.755[c]
Indication for PPV, *n* (%)				
Vitreous hemorrhage	4 (80.0)	23 (88.5)	-	0.525[a]
Fibrovascular membrane	3 (60.0)	17 (65.4)	-	1.000[a]
Tractional retinal detachment	4 (80.0)	18 (30.8)	-	1.000[a]

[a]Determined by Fisher exact test; [b]Determined by one-way analysis of variance; [c]Determined by independent-sample *t* test, with $P<0.05$ considered statistically significant.

Table 2 Concentrations of cytokines in vitreous fluid of patients in each group median (range)

Cytokines	No IVI group	IVI group	Control group
VEGF (pg/mL)	140.01 (122.62-208.32)	22.73 (17.00-28.85)	3.88 (3.00-8.27)
bFGF (pg/mL)	24.40 (8.64-71.46)	31.95 (19.73-49.32)	3.05 (1.35-7.39)
PN (ng/mL)	11.72 (1.79-15.13)	6.34 (1.97-17.94)	0.85 (0.57-1.23)
IL-6 (pg/mL)	48.06 (34.39-186.93)	58.74 (28.37-94.73)	12.77 (2.29-22.01)
IL-8 (pg/mL))	51.78 (27.52-129.92)	183.81 (94.71-310.79)	25.96 (6.75-60.15)
TNF-α (pg/mL)	4.01 (2.00-5.50)	3.11 (2.58-3.51)	2.00 (1.93-2.43)

VEGF: Vascular endothelial growth factor; IVI: Intravitreal anti-VEGF injections; bFGF: Basic fibroblast growth factor; PN: Periostin; IL: Interleukin; TNF: Tumor necrosis factor.

The concentrations of IL-6 and IL-8 in the ≥7-day group were both significantly higher than in the 3-day group (P=0.019, P=0.014, respectively), whereas no significant differences in IL-6 and IL-8 concentrations were observed between the 5-day group and ≥7-day group (both $P>0.05$), as well as between the 3-day group and 5-day group (both $P>0.05$; Figure 2).

Correlation of Vitreous Fluid Cytokine Concentrations and Degree of Vitreoretinal Fibrosis There was no difference in the degree of vitreoretinal fibrosis between the no IVI PDR group and IVI PDR group (χ^2=1.143, P=0.911). When comparing the IVI PDR subgroups in terms of vitreoretinal fibrosis, a statistically significant difference was found

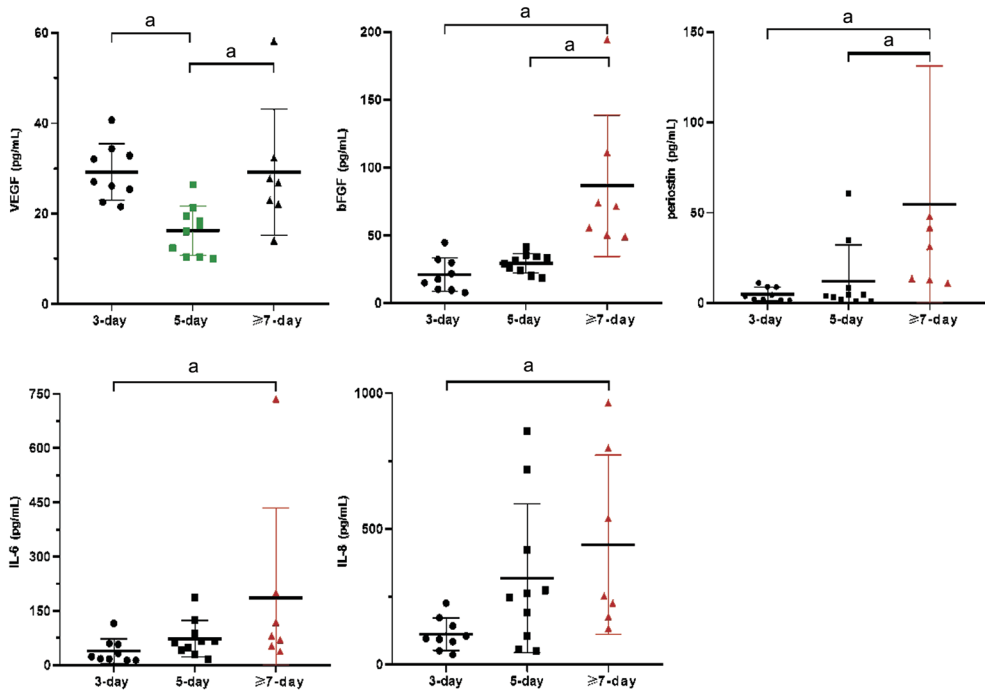

Figure 2 Concentration of cytokines in vitreous fluid of patients pre-treated with IVI at different time periods prior to PPV VEGF: Vascular endothelial growth factor; IVI: Intravitreal anti-VEGF injections; bFGF: Basic fibroblast growth factor; IL: Interleukin; TNF: Tumor necrosis factor. [a]*P*<0.05 by Kruskal-Wallis *H* test.

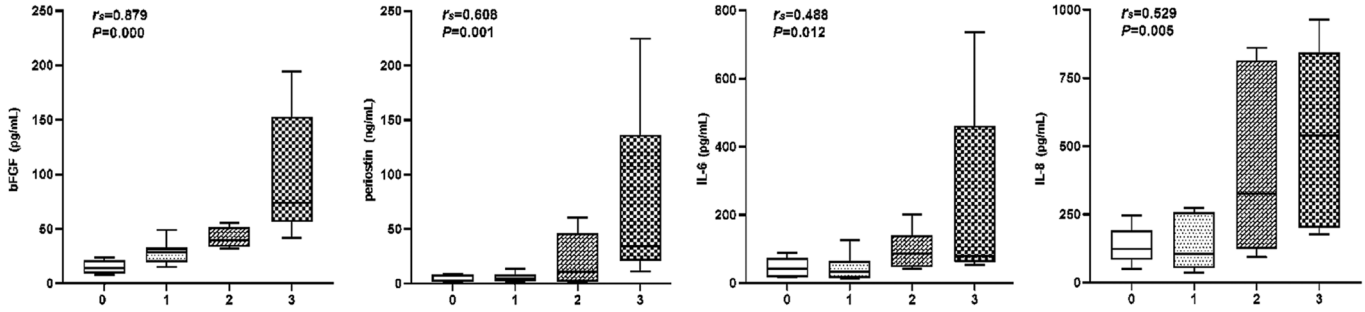

Figure 3 Correlation between the degree of vitreoretinal fibrosis and the concentration of cytokines in patients after intravitreal injection of anti-VEGF drugs VEGF: Vascular endothelial growth factor; bFGF: Basic fibroblast growth factor; IL: Interleukin.

(*Z*=8.988, *P*=0.011). No patient in the 3-day group exhibited vitreoretinal fibrosis of grade 3; on the contrary, in the ≥7-day group, vitreoretinal fibrosis of grade 3 occurred in 57% (4/7) of cases. Overall, the degree of vitreoretinal fibrosis increased significantly after IVI in the ≥7-day group compared to the 3-day group (*P*=0.015) and the 5-day group (*P*=0.039; Table 3).

For the primary outcome, the correlation between the degree of vitreoretinal fibrosis and the concentration of cytokines was analyzed by Spearman rank correlation test. In the IVI group, our analysis revealed that the degree of vitreoretinal fibrosis and the bFGF, PN, IL-6, and IL-8 concentrations correlated significantly (all *P*<0.05), with a Spearman's value of 0.879, 0.608, 0.488, and 0.529, respectively. On the contrary, the degree of vitreoretinal fibrosis did not correlate with the intravitreal concentration of VEGF (*P*=0.373) and TNF- α (*P*=0.587; Figure 3).

Table 3 Comparison of the degree of fibrosis in patients pre-treated with IVI at different time periods prior to PPV

Group	Degree of vitreoretinal fibrosis (*n*)				*Z*	*P*
	Grade 0	Grade 1	Grade 2	Grade 3		
3-day	3	4	2	0	8.988	0.011
5-day	3	4	2	1		
≥7-day	0	1	2	4		

IVI: Intravitreal anti-VEGF injections; PPV: Pars plana vitrectomy.

Univariate ordinal regression analysis to determine predictors of vitreoretinal fibrosis showed that bFGF, PN, IL-6, and IL-8 concentrations had similar associations with the degree of vitreoretinal fibrosis in the IVI PDR groups (all *P*<0.05). In a multivariate model with bFGF, PN, IL-6, and IL-8 concentrations as predictors of vitreoretinal fibrosis, bFGF was an independent risk factor for the progression of fibrosis in PDR patients pre-treated with IVI (Table 4).

Table 4 Ordered logistic regression analysis of vitreoretinal fibrosis

Variables	β	S.E.	Wald	P	OR	95%CI
Univariate						
bFGF	0.182	0.050	12.995	0.000	1.199	0.083-0.281
PN	0.079	0.030	7.130	0.008	1.083	0.021-0.138
IL-6	0.017	0.008	4.271	0.039	1.017	0.001-0.033
IL-8	0.005	0.002	7.390	0.007	1.005	0.001-0.008
Multiple						
bFGF	0.171	0.052	10.999	0.001	1.187	0.070-0.272

OR: Odds ratio; CI: Confidence interval; bFGF: Basic fibroblast growth factor; PN: Periostin; IL: Interleukin.

DISCUSSION

The development of diabetic retinopathy (DR) into advanced proliferative lesions involves a series of complicated pathological changes. From the macroscopic point of view, it is mainly the pathological changes of retinal micro-vessels, while from the microscopic, molecular, biological level, the increase or decrease of different kinds of cytokines in the eye and changes in the retinal immune microenvironment mediated by these cytokines accelerate the pathological progression of PDR. Changes in VEGF concentrations are the key event, mainly because VEGF is an important factor in retinal vascular leakage and pathological neovascularization. In this pilot study, significantly higher concentrations of VEGF were detected in the vitreous samples of PDR patients who were not pretreated with IVI compared to non-diabetic controls. In the last 10y, targeted inhibition of VEGF has been widely applied clinically as adjuvant therapy for PDR, especially as preoperative IVI prior to PPV[8-9].

Consistent with previous reports, we found that concentrations of VEGF were significantly lower in PDR patients pretreated with IVI. As VEGF inhibitors, anti-VEGF drugs prevent VEGF from binding to VEGF receptors such as vascular endothelial growth factor receptor-2 (VEGFR-2), thereby inhibiting endothelial cell proliferation and reducing vascular leakage, thus eliminating fragile neovascularization or inhibiting its formation[21-22]. A large number of studies have confirmed that preoperative IVI combined with PPV in the treatment of PDR can not only reduce intraoperative and postoperative complications, but also improve the visual prognosis of patients[11-13].

An important issue in combination therapy is the interval between PPV and IVI pretreatment. From the perspective of VEGF, it seems that the longer the interval time, the lower the VEGF concentration and the greater the advantage of combination therapy. Our study further compared the concentrations of VEGF in the vitreous at different time after anti-VEGF therapy, and found that VEGF concentrations decreased dramatically at 3, 5 and 7d after injection, and reached the lowest concentrations in patients who underwent PPV 5d after IVI. At present, reports on the changes in intraocular VEGF concentrations after IVI at different intervals are not consistent. Feng *et al*[23] proved that bevacizumab could reduce the concentration of VEGF in aqueous fluid of PDR patients and remaine low for 14d. Ma *et al*[24] reported that VEGF concentrations in vitreous fluid of PDR patients decreased significantly after intravitreal bevacizumab injection, and this low concentration remained for 4.4±2.2 to 34.8±33.7d, with the lowest value appearing within one week after injection. Judging from the above, there is no doubt that preoperative IVI can block intraocular VEGF levels, but this inhibitory effect is time-limited, so if clinicians want to improve the success rate and postoperative outcome of PPV in PDR patients, the timing of intravitreal injection of anti-VEGF before PPV should be chosen at the peak of drug action.

In the present study, we observed another important phenomenon: vitreoretinal fibrosis can develop or progress following IVI treatment, especially with a long interval between IVI and PPV. We compared the degree of vitreoretinal fibrosis after IVI at different times and found that grade 3 was dominant in the ≥7-day group, accounting for 57%, and the degree of vitreoretinal fibrosis was more serious than that in the 3-day group and 5-day group. El-Sabagh *et al*[25] showed that the longer the interval between intravitreal bevacizumab injection and PPV, the higher the degree of vitreoretinal fibrosis in eyes with PDR, while neovascularization in FVMs decreased significantly. It can be seen that vitreoretinal fibrosis will develop or progress with longer time intervals between IVI pretreatment and PPV; therefore, it is not advisable prolong the interval in clinic. Some prior studies have suggested that the above complications may be closely related to the angio-fibrotic switch[19-20,26], but the mechanism of this switch has not been elucidated.

Kuiper *et al*[20] discovered that the balance between connective tissue growth factor (CTGF) and VEGF determined the angio-fibrotic switch, and disruption of this balance could promote the transformation from angiogenesis to fibrosis in PDR patients. Combined with our study, one possibility is that preoperative IVI not only decreased VEGF but also caused

changes in the levels of other cytokines in PDR accordingly, which triggered the angio-fibrotic switch and disrupted the angio-fibrotic balance.

Our study further analyzed the changes in concentrations of other cytokines in the vitreous fluid of PDR patients after administration of anti-VEGF drugs at different times before PPV. We observed that intraocular concentrations of bFGF and periostin were highest in the ≥7-day group and were positively correlated with the degree of vitreoretinal fibrosis. In addition, the concentrations of IL-6 and IL-8 inflammatory cytokines in the ≥7-day group were significantly higher than those in the 3-day group. The role of bFGF and PN as pro-fibrotic cytokines is well-established in various diseases and recently in retinopathy as well[27-31]. IL-6 and IL-8 are members of the IL family involved in inflammation[32-33]. The changes in the above cytokine concentrations in this study can be related to a sharp and continuous decline in VEGF levels, which causes a compensatory increase of pro-fibrosis and inflammatory cytokine concentrations. When the level of these cytokines reaches a threshold value, especially pro-fibrosis factors, the angio-fibrotic switch will be triggered, which can further up-regulate the expression of pro-fibrosis cytokines. In addition, with the progression of vitreoretinal fibrosis, retinal hypoxia is aggravated, which leads to a certain degree of inflammation. Fibrosis and inflammation interact with each other, forming a vicious cycle. Therefore, the optimal timing of preoperative IVI treatment for PDR patients is when anti-VEGF drugs can minimize intraocular VEGF concentrations and do not trigger the angio-fibrotic switch. On the basis of our research, it is suggested that PPV should be performed 5d after IVI.

In addition, it is worth mentioning that multivariate ordered logistic regression analysis showed bFGF was an independent risk factor for the progression of vitreoretinal fibrosis in active PDR patients after IVI. This suggests that bFGF may be a new target for anti-fibrosis therapy, anti-bFGF combined with anti-VEGF drugs can be considered to assist PPV in the treatment of PDR in the future.

Our study has some limitations. First, our findings should be considered preliminary. However, our previous clinical study found that the degree of vitreoretinal fibrosis could be aggravated with prolonged interval between IVI and PPV. Such favorable outcomes require larger-scale clinical trials to be proven. Second, this study elucidated the mechanism of the "angio-fibrotic switch" only by detecting the concentration of cytokines in the vitreous fluid. Further studies with a larger sample size and immunohistochemical experiments of FVMs are needed to co-validate the data.

In summary, in PDR patients, the vitreous concentrations of a variety of cytokines will change after pretreatment with IVI before PPV. Prolonged blocking of VEGF cytokine may trigger the "angio-fibrotic switch". We suggest that PPV be performed on the fifth day after the administration of anti-VEGF drugs in PDR patients because VEGF concentrations in vitreous fluid reached the lowest level, and concentrations of bFGF and PN have not increased significantly. Meanwhile, the degree of vitreoretinal fibrosis can significantly increase in patients with a pretreatment interval ≥7d prior to PPV, which is mainly related to the significantly increased concentrations of bFGF, PN, IL-6, and IL-8 in the vitreous fluid. bFGF is an independent risk factor for fibrosis progression and is expected to become a new target for anti-fibrosis therapy.

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