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Serum and vitreous vascular endothelial growth factor levels in diabetic retinopathy

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Abstract

Purpose To research whether serum vascular endothelial growth factor (VEGF) levels could be used to evaluate diabetic retinopathy (DR) progression and to compare vitreous VEGF levels after injections of intravitreal bevacizumab (IVB), ranibizumab (IVR), and triamcinolone acetonide (IVTA) in proliferative diabetic retinopathy (PDR).

Methods We enrolled a total of 91 eyes of 89 subjects (70 eyes of 68 diabetics and 21 eyes of 21 non-diabetic controls). The diabetic subjects were divided into three groups as PDR ($n=28$), non-proliferative diabetic retinopathy ($n=20$), and no-DR ($n=20$). Eyes with PDR ($n=31$) were injected with IVB ($n=7$), IVR ($n=10$), or IVTA ($n=6$) 3 days before vitrectomy, and eight eyes did not receive an injection. Serum and vitreous samples were collected before vitrectomy and analyzed using ELISA.

Results We found the severity of retinopathy was not correlated with serum VEGF levels ($P=.919$, $\rho=-0.011$). Compared with the controls, vitreous VEGF was higher in the PDR ($P<.001$), whereas serum VEGF did not differ ($P=.99$). The controls had lower vitreous VEGF than the IVB, IVR, and no-injection subgroups ($P=.01$, $P<.001$, and $P=.04$, respectively). Vitreous VEGF was similar among the injected and no-injection subgroups ($P=.17$).

Conclusions Serum VEGF levels may not directly reflect retinopathy progression. Neither IVB, IVR nor IVTA could eliminate vitreous VEGF levels within 3 days before vitrectomy.

Keywords Bevacizumab · Diabetic retinopathy · Ranibizumab · Triamcinolone acetonide · Vascular endothelial growth factor · Vitreous humor

Introduction

Diabetic retinopathy (DR) as an ocular adverse effect of diabetes mellitus (DM) is a potentially blinding condition in adults aged 20–74 years [1]. Proliferative diabetic retinopathy (PDR), which can progress with neovascularization and fibrovascular proliferation on the vitreoretinal surface, is the advanced form of DR [2]. PDR is present in 1.9–4.2% of individuals with DM [3] and a series of studies showed that significantly higher vascular endothelial growth factor (VEGF) levels are associated with PDR [4].

In PDR, elevated vitreous VEGF levels were found compared with non-proliferative diabetic retinopathy

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(NPDR) [5]. However, there are discordant outcomes regarding serum VEGF levels. Some studies indicated higher serum VEGF levels [6], whereas others reported that serum VEGF levels were not necessary to assess PDR [7].

Additionally, in the treatment of PDR, the use of VEGF antagonists as preoperative therapy for pars plana vitrectomy (PPV), such as an injection of intravitreal bevacizumab (IVB) [8, 9] and intravitreal ranibizumab (IVR), is recommended as a safe and effective method [10]. It was also reported that intravitreal triamcinolone acetonide (IVTA) reduces worsening macular thickening in PDR [11].

Although uncontrolled blood glucose levels, dyslipidemia, and high blood pressure are risk factors for DR in diabetes, only a small portion of retinopathies has been associated with these factors [12]. Therefore, additional investigations are needed to reveal the mechanisms of DR progression.

We aimed to investigate whether serum VEGF levels could be used to evaluate PDR progression indirectly because vitreous VEGF levels are challenging to measure. The primary objective of our study was to assess whether serum VEGF was associated with retinopathy progression and investigate the correlations between vitreous and serum VEGF levels in PDR. As a secondary objective, we aimed to compare serum and vitreous VEGF levels after applying IVB, IVR, or IVTA to eyes with PDR.

This study is thought to be significant due to the previously limited literature knowledge about IVTA injections performed before PPV. We could not find any other study which simultaneously compared the mentioned adjuvants administered before vitrectomy.

Methods

Patients

This was a randomized, controlled study in which patients with diabetes and non-diabetic controls were recruited from the Department of Ophthalmology, Marmara University, the School of Medicine, Istanbul, Turkey, between November 2014 and May 2015. The main inclusion criteria for the patients with diabetes were age (≥18 years) and having type 2 DM, and for non-diabetic control patients having an epiretinal membrane, a macular hole, or a rhegmatogenous retinal detachment without DM. The exclusion criteria were as follows: having systemic disorders that might affect serum or vitreous VEGF levels such as malignancy or history of chemotherapy, renal diseases causing high serum creatinine levels above 1.5 mg/dL, alcohol or smoking addiction, hematologic or hepatologic disorders, and having ocular problems such as senile macular degeneration, retinal vein occlusion, active or chronic uveitis, history of PPV or panretinal photocoagulation in the last two months.

Ninety-one eyes of 89 subjects (68 patients with type 2 DM and 21 non-diabetic controls) were randomly enrolled. Detailed eye examinations including best-corrected visual acuity (Snellen chart), biomicroscopic anterior segment slit-lamp examinations, and intraocular pressure measurements using a non-contact pneumotonometer were performed on all participants. After the pupils were dilated with phenylephrine and tropicamide eye drops, dilated fundus examinations were performed. When necessary, fundus fluorescein angiography of patients with diabetes was obtained using a TopconTRC-50DX fundus camera (Topcon, Tokyo, Japan). Macular images were obtained using optical coherence tomography (OCT) (Optovue Inc., Fremont, CA, USA). Patients with diabetes were divided into three groups; no DR ($n=20$), NPDR ($n=20$), and PDR ($n=28$), in line with the Early Treatment of Diabetic Retinopathy Study [13]. Eyes in the PDR group had the diagnosis of tractional retinal detachment, intravitreal hemorrhage, or both. The non-diabetic control group included eyes with a macular hole, epiretinal membrane, or retinal detachment that underwent PPV. During PPV, 52 vitreous samples were collected from 31 eyes of 28 patients with PDR and 21 eyes of 21 controls. Eyes with PDR received either IVB, IVR, or IVTA 3 days before PPV as part of the standard procedure.

This study was approved by Marmara University, Faculty of Medicine, Ethics Committee for Clinical Research, Istanbul, following the Helsinki Declaration. Written informed consent was taken from the participants. Additionally, this project was supported by the Marmara University Scientific Research Projects Committee (BAPKO).

Serum sampling and preservation

After eight hours of fasting, blood samples were collected from the antecubital vein into vacutainer tubes either with a gel separator or K_2EDTA (Becton Dickinson, USA). In the PDR and control groups, samples were taken while beginning the vitrectomy before applying any anesthetic agent. Glycosylated hemoglobin (HbA1c) levels were measured in EDTA-anticoagulated whole blood samples chromatographically (Bio-Rad VARIANT-II). First, gel separator tubes were centrifuged at 2000×*g* for 10 min., and then, the obtained samples were used for routine examinations. Glucose, total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen, and creatinine were tested spectrophotometrically (Beckman Coulter, AU5800). For VEGF measurements, second tubes were centrifuged at 1000×*g* for 15 min at 4°C, and 200 µL aliquots were stored at −80°C until required for analysis.

Surgical procedures, vitreous sampling, and preservation

All vitreous samples were collected from eyes during PPV under general anesthesia. All eyes underwent either 20 or 23-gauge standard three-port PPV. Undiluted vitreous samples (range, 0.5–1 mL) were obtained using an injector-attached vitrectomy probe (Xenon Bright Star Illumination System, D.O.R.C. Netherlands). During the collection, no fluid leakage was allowed into the eye by turning the infusion probe to the air mode. Tubes containing vitreous samples were kept on ice, and they were immediately transferred to the laboratory and kept there at −80°C until required for analysis. No complications related to PPV were observed.

Intravitreal bevacizumab, ranibizumab, or triamcinolone acetonide injection in PDR

In 31 eyes with PDR, a total of 23 eyes were injected with either 1.25 mg/0.05 mL IVB ($n=7$, Avastin 100 mg/4 mL, Roche), or 1 mg/0.1 mL IVR ($n=10$, Lucentis 10 mg/1 mL, Novartis) or with 4 mg/0.1 mL IVTA ($n=6$, Kenakort–A 40 mg/mL, Deva) 3 days before PPV. No injections were performed on eight eyes. Eyes were injected 3.5 to 4 mm behind the corneal limbus. No complications such as uveitis, endophthalmitis, retinal detachment, ocular hypertension, or any systemic adverse events occurred related to intravitreal injections.

Vitreous processing and analysis

Enzyme-linked immunosorbent assay (ELISA) was used for the analysis of vitreous and serum VEGF levels. The assay was performed using a human VEGF-A platinum ELISA kit in line with the instructions (BMS277/2, eBioscience). To prevent the effects of serum diffusion due to the breakup of the blood-retinal barrier, vitreous VEGF levels were normalized by dividing by the vitreous total protein concentration. The vitreous total protein levels were measured automatically (AU680, Beckman Coulter, USA). In the data provided by the manufacturer, the intrinsic variability coefficients were determined as 1.7, 1.2, and 0.7% for concentrations of 15, 53, and 152 mg/dL, respectively. The coefficients of variability between days were determined as 4.8, 1.9, and 1.7%, respectively, for the same concentrations (measurement range 4–200 mg/dL).

Statistical analysis

The statistical significance level was set to ≤ 0.05. Normality of data distribution was investigated using the Shapiro–Wilk test for all numerical variables. The relationship between categorical variables was analyzed using the Chi-square test. The Kruskal–Wallis and Mann–Whitney *U* tests were used to understand the differences between independent groups. Dunn's tests were used for post hoc analyses. Correlation analysis was calculated using Spearman's rank correlation tests.

A computer statistical power analysis program (G*Power 3, Heinrich Heine University, Düsseldorf, Germany) was applied to calculate the sample size with a strength of 0.90 and an α error of 0.05.

Results

The basic characteristics of diabetic and control groups are given in Table 1. No statistically significant difference was found in age, sex, body mass index, the presence of hypertensive and coronary artery diseases ($P=0.154$, $P=0.411$, $P=0.543$,

Table 1 Patient characteristics

Characteristic	Control ($n=21$)	Diabetic groups (n:68)			
		No DR ($n=20$)	NPDR ($n=20$)	PDR ($n=28$)	p value
Age (Median, IQR)	60 (20)	56.5 (15.3)	62 (14.5)	53 (15)	.154*
Gender (n, %)					
Female	10 (47.6)	8 (40)	6 (30)	15 (53.4)	.411†
Male	11 (52.4)	12 (60)	14 (70)	13 (46.6)	
BMI (kg/m^2) (Median, IQR)	29.4 (6.3)	31.2 (5.8)	30.3 (7.8)	29.9 (4.2)	.543*
HT (n, %)					
Absent	11 (52.4)	10 (50)	7 (35)	8 (28.6)	.274†
Present	10 (47.6)	10 (50)	13 (65)	20 (71.4)	
CAD (n, %)					
Absent	19 (90.5)	18 (90)	19 (95)	22 (78.6)	.395†
Present	2 (9.5)	2 (10)	1 (5)	6 (21.4)	
HbA1c (Median, IQR)	–	7.8 (2.8)	8.5 (2.1)	8.2 (2)	.241*
Duration of diabetes (Median, IQR)	–	12 (7.5)	16.5 (10.3)	16 (8)	.134*

no DR no diabetic retinopathy, *NPDR* non-proliferative diabetic retinopathy, *PDR* proliferative diabetic retinopathy, *PRP* panretinal photocoagulation, *BMI* body mass index, *CAD* coronary artery disease, *HT* hypertension, *n* number of patients, *IQR* interquartile range

*Kruskal–Wallis test

†Chi-square test

$P=0.274$ and $P=0.395$, respectively). HbA1c and duration of diabetes were not significantly different among the three diabetic groups ($P=0.241$ and $P=0.134$, respectively).

Three of the 28 patients with PDR underwent PPV on both eyes, and the intervals between the two operations were 4, 4.5, and 6 months. Serum samples, as well as vitreous samples of those patients, were collected simultaneously during the surgical procedure. As a result, a total of 31 serum and 31 vitreous samples from 31 eyes of 28 patients with PDR were obtained (Table 2).

Serum VEGF levels were not significantly different between the total diabetic and control groups (642.1 pg/mL vs. 358.1 pg/mL; $P=0.153$). The results were similar after adjusting for total protein levels (9.1 pg/mg vs. 5.1 pg/mg; $P=0.160$). By contrast, serum VEGF levels differed significantly

Table 2 Serum VEGF and total protein levels in diabetic and control groups

Serum levels	Control ($n=21$)	Diabetic groups (n:71)			p value
		No-DR ($n=20$)	NPDR ($n=20$)	PDR ($n=31$)	
Total protein (g/dL) (Median, IQR)	7.1 (0.9)	7.1 (0.6)	7.4 (0.5)	6.9 (0.9)	.026 ‡
VEGF (pg/mL) (Median, IQR)	358.1 (399.1)	596 (1025)	1133 (584.6)	363 (558.7)	.001*/†
VEGF / Total protein (pg/mg) (Median, IQR)	5.1 (5.5)	8.4 (13.0)	15.4 (7.5)	4.9 (8.7)	.003§/II

no DR no diabetic retinopathy, *NPDR* nonproliferative diabetic retinopathy, *PDR* proliferative diabetic retinopathy, *VEGF* vascular endothelial growth factor, *IQR* interquartile range

‡$P=.019$; PDR vs. NPDR

*$P=.001$, PDR vs. NPDR

†$P=.006$; control vs. NPDR

§$P=.003$; PDR vs. NPDR

II$P=.01$; control vs. NPDR Kruskal–Wallis test

in the diabetic groups ($P=0.001$), and the results appeared similar after adjustment with total protein levels ($P=0.003$). Post hoc tests showed that higher serum VEGF levels existed in the NPDR group when compared with those in the PDR and control groups ($P=0.001$ and $P=0.006$, respectively) (Table 2).

The characteristics of eyes in the PDR subgroups are shown in Table 3. This is a limitation of our study in that we did not measure pre-injection vitreous VEGF levels because of ethical problems. There was no statistical difference in the history of pan-retinal photocoagulation, indication for vitrectomy, or any other situation that may cause a difference in vitreous VEGF level. So in PDR pre-injection vitreous VEGF levels were considered to be similar.

The ratio of previous pan-retinal photocoagulation, which may cause a reduction of the VEGF levels, was not significantly different among the PDR subgroups ($P=0.46$). In hypoxic conditions, elevated vitreous VEGF levels are mainly due to intraocular synthesis. However, increased serum diffusion because of the break-up of the blood-retinal barrier could also contribute to this finding. To eliminate this contributory effect of the diffusion, vitreous protein concentration should be measured, and the findings were corrected as vitreous VEGF/total protein ratio (pg/mg). [14]

Our study found higher vitreous VEGF levels in the PDR group than in the control group (291.1 pg/mL vs. 35.4 pg/mL; $P<0.001$). The results were also higher after correction with total protein levels (170.8 pg/mg vs. 30.9 pg/mg; $P<0.001$). However, serum VEGF levels did not differ between PDR and control groups (358.1 pg/mL vs. 363 pg/mL; $P=0.99$). No correlation was detected between vitreous and serum VEGF levels in the PDR group ($P=0.35$, $\rho=0.17$) (Table 4).

Significantly different vitreous VEGF levels and VEGF/ total protein ratio were determined among the PDR subgroups and controls ($P<0.001$ and $P=0.003$, respectively). In the eyes in the control group, lower vitreous VEGF levels were found compared with those of IVB, IVR, and no injection eyes in the PDR group ($P=0.01$, $P<0.001$, and $P=0.04$,

Table 3 The characteristics of proliferative diabetic retinopathy

Characteristics	No injection ($n=8$)	IVB ($n=7$)	IVR ($n=10$)	IVTA ($n=6$)	p value
Age (Median, IQR)	62.5 (12.8)	51 (20)	51 (8.3)	50.5 (11.3)	.172*
Gender (n, %)					
Female	5 (62.5)	3 (42.9)	5 (50)	3 (50)	.925†
Male	3 (37.5)	4 (57.1)	5 (50)	3 (50)	
BMI (kg/m^2) (Median, IQR)	30 (4.8)	29.8 (2.6)	30.9 (6.1)	29.1 (5.6)	.730*
HT (n, %)					
Absent	2 (25)	3 (42.9)	2 (20)	3 (50)	.577†
Present	6 (75)	4 (57.1)	8 (80)	3 (50)	
CAD (n, %)					
Absent	7 (87.5)	6 (85.7)	7 (70)	5 (83.3)	.867†
Present	1 (12.5)	1 (14.3)	3 (30)	1 (16.7)	
HbA1c (Median, IQR)	8.3 (3.4)	7.4 (2.1)	7.7 (2.8)	8 (1.4)	.870*
Duration of diabetes (Median, IQR)	21 (16.8)	16 (3)	14 (6.5)	15.5 (7)	.266*
History of PRP (n, %)	5 (62.5)	3 (42.9)	7 (70)	2 (33.3)	.465†
Indication for PPV (n, %)					
TDR	3 (37.5)	3 (42.8)	5 (50)	2 (33.3)	
IVH	0 (0)	1 (14.3)	1 (10)	1 (16.7)	.944†
TDR + IVH	5 (62.5)	3 (42.8)	4 (40)	3 (50)	

PRP panretinal photocoagulation, *BMI* body mass index, *CAD* coronary artery disease, *HT* hypertension, *PPV* pars plana vitrectomy, *IVH* intravitreal hemorrhage, *TRD* tractional retinal detachment, *IQR* interquartile range

*Kruskal–Wallis test

†Chi-square test

Table 4 Vitreous VEGF levels in PDR subgroups and control group

Serum and vitreous levels	PDR (*n*: 31)					*p* value
	Control (*n* = 21)	No injection (*n* = 8)	IVB (*n* = 7)	IVR (*n* = 10)	IVTA (*n* = 6)	
Serum						
Total protein (g/dL) (Median, IQR)	7.1 (0.9)	6.6 (1.0)	6.9 (0.6)	7.5 (1.0)	6.9 (0.8)	.423
VEGF (pg/mL) (Median, IQR)	358 (399)	328 (547)	130 (145)	602 (633)	346 (845)	.255
VEGF / Total protein (pg/mg) (Median, IQR)	5.1 (5.5)	5.1 (8.5)	1.9 (1.8)	8.0 (9.0)	4.9 (13.1)	.302
Vitreous						
Total protein (mg/dL) (Median, IQR)	111 (198)	177 (373)	166 (142)	160 (476)	217 (239)	.369
VEGF (pg/mL) (Median, IQR)	35.4 (101)	216 (616)	326 (982)	519 (1190)	130 (428)	<.001*†‡
VEGF / Total protein (pg/mg) (Median, IQR)	30.9 (89.7)	132 (278)	337 (434)	320 (671)	92.5 (541)	.003

IVB intravitreal bevacizumab, *IVR* intravitreal ranibizumab, *IVTA* intravitreal triamcinolone acetonide, *VEGF* vascular endothelial growth factor, *PDR* proliferative diabetic retinopathy, *IQR* interquartile range

*$P = .01$, control vs. IVB

†$P < .001$, control vs. IVR

‡$P = .04$, control vs. no injection Kruskal–Wallis test

respectively). Vitreous VEGF levels were similar among the PDR subgroups (IVB, IVR, IVTA, or no injection eyes) ($P = 0.17$). After adjusting for total protein levels, similar results were found ($P = 0.42$). Serum VEGF levels did not differ among the injected PDR subgroups ($P = 0.25$).

Discussion

Serum VEGF levels in patients with diabetes and controls

This study found no difference in serum VEGF levels between total diabetic and control groups ($P = 0.153$). Serum VEGF levels in the control group were similar to the PDR and no DR groups ($P = 0.99$ and $P = 0.99$, respectively). The highest serum VEGF levels were observed in the NPDR group compared with the PDR and control groups ($P = 0.001$ and $P = 0.006$, respectively).

There is no consensus about the effect of serum VEGF levels on DR. A meta-analysis demonstrated elevated serum VEGF levels in patients with NPDR and PDR compared with those with non-diabetic retinopathy [15]. Another article noted higher serum VEGF levels in the PDR group compared with non-diabetic retinopathy, NPDR, and control groups [6]. Ozturk et al. reported serum VEGF levels were similar between the NPDR and PDR groups and elevated serum VEGF in these groups compared with healthy controls [16].

In some studies, serum VEGF levels did not differ among patients with PDR, NPDR, no DR [17, 18], and healthy controls [7, 19]. Other studies detected similar plasma VEGF levels between patients with PDR and controls, though there was an elevated vitreous VEGF concentration in patients with PDR compared with controls [20]. Consistent with our results, Mesquita et al. found higher serum VEGF-A and VEGF-B levels in patients with NPDR when compared with patients with PDR [21]. Whether higher concentrations of serum VEGF may be a specific biomarker for NPDR, or possible inhibitory effects of anti-diabetic agents used for strict control of blood glucose on VEGF production in advanced stages of diabetes should be further investigated. Although serum VEGF elevation in NPDR is not entirely clear, it may be a marker of the onset of uncontrolled diabetes.

Vitreous VEGF levels in patients with PDR and controls

We have found, as expected, elevated vitreous VEGF in patients with PDR compared with the controls

($P<0.001$). The results were similar after adjusting the results for total protein levels ($P<0.001$). In agreement with our results, a study [22] found higher vitreous VEGF levels in eyes with PDR (585.67 pg/mL) compared with controls (123.85 pg/mL) ($P<0.001$). Other studies are reporting similar findings [23, 24]. Comparable with our results, some other studies [14] reported high VEGF and vitreous VEGF/ vitreous total protein ratio in subjects with PDR when compared with controls.

The correlation between serum and vitreous VEGF levels

There was no correlation between serum and vitreous VEGF levels in the PDR group ($P=0.35$, $\rho=0.17$). Serum VEGF and severity of retinopathy were not correlated either ($P=0.919$, $\rho=-0.011$). However, vitreous VEGF levels were correlated with vitreous total protein in the PDR group ($P=0.031$, $\rho=0.388$), in contrast to the control group ($P=0.907$, $\rho=-0.027$).

Consistent with our study, Burgos et al. [25] demonstrated that vitreous VEGF levels were not affected by its serum levels in PDR. Another study reported that serum VEGF was not correlated with the progression of diabetic retinopathy [17]. Muhiddin et al. [26] showed significantly elevated vitreous VEGF-A in patients with PDR compared with controls, despite the existence of no differences in their serum levels. In another study, vitreous VEGF-A levels were found higher in patients with diabetes compared with controls; however, their correlation with plasma VEGF was not detected [20].

In our study, although higher vitreous VEGF, as well as higher vitreous VEGF/total protein ratio, was found in patients with PDR than in the controls ($P<0.001$ for both), serum VEGF levels were similar between the two groups ($P=0.99$). In PDR, vitreous and serum VEGF levels were not correlated ($P=0.35$, $\rho=0.17$). These results strongly suggest that the vitreous VEGF levels cannot be predicted from serum VEGF levels in PDR.

The effects of triamcinolone acetonide, bevacizumab, and ranibizumab

To our knowledge, there has been no comparative study evaluating vitreous VEGF levels 3 days following intravitreal bevacizumab, ranibizumab, or triamcinolone acetonide injections. In the current study, vitreous VEGF levels in the control group were significantly lower than those of the IVB, IVR, and no-injection eyes. However, vitreous VEGF levels did not differ among the IVB, IVR, IVTA, and no injection groups.

a. Triamcinolone acetonide

There are few reports regarding the use of IVTA as an adjunct to PPV. In a study comparing vitreous VEGF levels 7 days following IVB (19 eyes) and IVTA (10 eyes), IVB resulted in the lowest vitreous VEGF compared with IVTA and non-injected diabetic eyes ($P<0.001$ for both). Consistent with our study, vitreous VEGF concentrations were similar in eyes with IVTA and non-injected eyes ($P>0.05$) [14]. Another study reported that VEGF could be eliminated 1 month after IVTA in DR [11]. It seems that 3 days may not be sufficient for IVTA to suppress vitreous VEGF in eyes with PDR.

b. Bevacizumab and ranibizumab

The current study showed lower vitreous VEGF levels in non-diabetic controls compared with the IVB, IVR, or non-injected groups. A recent study reported similar vitreous VEGF-A levels in patients with diabetes compared with non-diabetic controls after an average of 55.8 (range, 18 to 88) days of anti-VEGF injection [20]. Another study demonstrated, in PDR, lower aqueous VEGF levels on the second ($P=0.011$) and the fifth day following IVB injection compared with the non-injected eyes ($P=0.012$). The authors recommended that PPV should be performed within 1 week after IVB injection in PDR [27]. Vitreous VEGF levels were lower after 7 days compared with within 7 days of IVB injection ($P=0.035$) [28]. Other studies reported decreased vitreous VEGF levels within 7 days of IVB injection [14, 29]. Another study with IVR confirmed that 3 to 7 days were enough to reduce vitreous VEGF levels, but the lowest value was obtained after 7 days when it was used as adjunctive therapy before PPV for PDR ($P=0.041$) [30]. Our results showed that both IVB and IVR could not decrease vitreous VEGF levels within 3 days in eyes with PDR. VEGF suppression with anti-VEGF agents may be more prominent after 7 days. Although

prior injections of IVB, IVR, and IVTA allowed us to perform PPV safely, in clinical practice, it might be necessary to wait a little longer to establish their anti-VEGF effect. An increased number of subjects may alter the results.

In conclusion, elevated serum VEGF levels may not indicate retinopathy progression. Although it is easier to measure serum VEGF levels, it can be concluded that vitreous VEGF determination cannot be made by evaluating serum VEGF levels in clinical practice. Compared with controls, patients with diabetics had elevated vitreous VEGF levels. Intravitreal injections of bevacizumab, ranibizumab, and triamcinolone acetonide were ineffective in reducing vitreous VEGF levels within 3 days.

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Author contributions S-D and C designed the study. C performed the operations. H worked on biochemical analyses. S-D wrote the main manuscript text. S-D prepared tables. All authors (S-D, C, and H) analyzed and reviewed the manuscript.

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Declarations

Competing interests The authors declare no competing interests.

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