

## Findings of a study of pigment epithelium-derived factor (PEDF) levels in vitreous samples from patients with proliferative diabetic retinopathy

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**Key words:** proliferative diabetic retinopathy, pigment epithelium-derived factor, electrophysiological examination

**Purpose:** To investigate the relationship, if any, between vitreous pigment epithelium-derived factor (PEDF) levels and a number of clinical factors used to assess the functional activity of the retina after an intravitreal surgery for complications of proliferative diabetic retinopathy (PDR).

**Materials and Methods:** Seventy PDR patients (70 eyes) were involved into the study. PEDF levels in vitreous samples were quantified by enzyme-linked immunosorbent assay.

**Results:** In vitreous samples from eyes with PDR the mean  $\pm$  SD PEDF level was 3.26 ng/mL  $\pm$  1.57 ng/mL. Vitreous PEDF levels were statistically significantly higher for eyes operated on for vitreous hemorrhage (VH) only than for eyes operated on for VH in the presence of macular traction detachment ( $t=2.35$ ,  $p=0.022$ ). The lower was the vitreous PEDF level (lower median value, 3.12 ng/mL), the more severe was retinal ischemia, which was confirmed by electrophysiological examination (namely, the parameters of the oscillatory potentials). It was found that when the level of PEDF in the vitreous is decreased, retinal neurodegeneration develops, with the greatest damage exhibited by bipolar, amacrine, and Muller cells.

**Conclusions:** Parameters of photopic and 30 Hz flicker ERG as well as of oscillatory potentials are surrogate indicators of levels of PEDF in ocular tissues, and may be used in later studies for investigation of the role of PEDF levels in pathological processes in the eye.

Diabetic retinopathy (DR) is a major cause of blindness worldwide, and its prevalence in the USA is expected to reach 3.4 million in 2050 [6]. In Ukraine, the annual incidence rate of DR has been rising steadily in recent decades, and increased from 1.2 per 10,000 in 1975 to 15.2 per 10,000 in 2000 and to as much as 19.5 per 10,000 in 2010 [2].

Ischemia is one of the major pathogenetic mechanisms which underlie ocular angiogenesis and proliferation in diabetes mellitus (DM), resulting in the development of vitreous hemorrhage (VH) and epiretinal membranes (EM), followed by that of traction retinal detachment.

Pigment epithelium-derived factor (PEDF) is a potential angiogenic inhibitor and has potent anti-inflammatory properties. Decreased vitreous levels of PEDF result in abruptly increased vascular endothelial growth factor (VEGF) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) levels and are associated with activation of inflammation and elevated vascular permeability [8, 15].

PEDF has been reported to maintain cell viability in cultured retinal neurons through attenuation of hydrogen peroxide- and reactive oxygen-induced apoptosis [5, 7]. In addition, in a model of photoreceptor dysmorphogenesis induced by removal of the retinal pigment epithelium, this neurotrophic factor has been shown to maintain photoreceptor cell survival even after exposure to damaging light [9, 12].

Serum PEDF levels in DM are known to decrease gradually as DR advances, and to be statistically significantly lower in proliferative diabetic retinopathy (PDR) than in non-proliferative disease. No significant change in serum PEDF levels and a significant reduction in serum VEGF levels have been observed in PDR patients after vitrectomy [10].

In a limited number of studies it has been shown that vitreous PEDF levels in PDR are statistically significantly higher than those in a macular tear and in rhegmatogenous retinal detachment [4, 11].

However, the literature is scarce on the relationship between vitreous PEDF levels and the stage of proliferation in eyes with PDR. Such data may contribute significantly to the understanding of the development of retinal neurodegeneration in patients with this disorder.

The study purpose was to investigate the relationship, if any, between vitreous PEDF levels and a number of clinical factors used to assess the functional activity of the retina after an intravitreal surgery for complications of PDR.

### Materials and Methods

Seventy patients (70 eyes) who had undergone intravitreal interventions for complications of PDR with a successful anatomic result were included into this study and underwent examination at about 2 months after surgery. There were 32 men and 38 women, of whom 12 subjects (17.1%) and 58 subjects (82.9%) had type I and type II diabetes, respectively. Mean age was 55.7 years (SD, 12.7 years), and mean diabetes duration was

18 years (SD, 7.4 years). The numbers (percentages) of patients with adequately and sub-adequately controlled DM were 50 (71.4%) and 20 (26.8%), respectively. The level of diabetes control was assessed using daily variations in blood glucose level, and presence or absence of glucose and protein in urine. Eighteen patients (31.4%) were hypertensive. The interval from the first sign of proliferative disease till examination varied from 10 months to 32 months with mean value of 17.2 months (SD, 5.8 months). In 62 (88.6%) eyes, vitrectomy was performed after the patient's neovascularization had proved unresponsive to panretinal laser coagulation. The intraocular pressure (IOP) was within the normal range in all eyes. Indications for vitrectomy were VH without EM in 35 (50.0%) eyes, partial or total VH in the presence of macular traction detachment (MTD) in 28 (40.0%) eyes, and combined traction-rhegmatogenous retinal detachment (TRD/RRD) in 7 (10.0%) eyes.

Intravitreal interventions were performed using 23G instrumentation. In brief, a core vitrectomy was followed by removal of EM (with removal of all islands of fibrovascular tissue), if any. Then, fluid air exchange for retinal folds was performed, and panretinal coagulation and coagulation of retinal breaks with endodiode laser were performed, if required. Surgeries were completed with intraocular tamponade using sterile air in 8 (11.4%) eyes and either 10% or 20% perfluoropropane in 62 (88.6%) eyes.

Baseline visual acuity (VA) in eyes operated on for VH only varied from light perception to 0.05 and, in the majority (27 (77.1%)) of them was 0.01. In eyes operated on for VH in the presence of MTD, it varied from light perception to 0.25 and, in the majority (20 (71.4%)) of them was 0.01 to 0.03. In eyes operated on for TRD/RRD, baseline VA varied from light perception to 0.35. A RETIScan System (Roland Consult, Wiesbaden, Germany) was used to perform electrophysiological tests according to the ISCEV Standards [13]. Scotopic (dim-flash) electroretinogram (ERG) and photopic (light-flash) ERG, representing pure rod response and cone function, respectively, were performed on a dark-adapted eye and on a light-adapted eye, respectively. Oscillatory potentials (OPs) were recorded under light-adapted conditions. The 30 Hz flicker ERG (the so-called rhythmic ERG) as well as mixed ERG (maximum response from the dark-adapted eye) was recorded [3].

At the initial phase of vitrectomy, with irrigation turned off, a 0.2-0.3  $\mu$ L vitreous sample were obtained from each study eye and placed into a container. The samples were stored at -20°C until analysis for PEDF levels. PEDF levels in vitreous samples were quantified by enzyme-linked immunosorbent assay kit (ELISA; R&D SYSTEMS, Minneapolis, MN) per the manufacturer's instructions. Photometric measurements were performed on an ELISA plate reader (Stat Fax-2100, Awareness Technologies Inc, Palm City, FL).

All statistical analyses were performed by STATISTICA 7 software (Statsoft, Tulsa, OK). Student's t-test and Chi-square test were used for comparison of variables as appropriate. A P-value less than or equal to 0.05 was considered to be statistically significant.

## Results and Discussion

In vitreous samples from eyes with PDR the mean  $\pm$  SD PEDF level was 3.26 ng/mL  $\pm$  1.57 ng/mL (range, 0.94 ng/mL to 7.57 ng/mL; median, 3.12 ng/mL).

The PEDF level in vitreous samples was 3.84 ng/mL in patients with DM type I and 3.13 ng/mL in patients with DM type II, with the difference being not statistically significant ( $t=1.43$ ,  $p=0.156$ ). Similarly, no statistically significant difference was found between hypertonic and non-hypertonic patients in terms of PEDF level in vitreous samples (3.18 ng/mL vs 3.47 ng/mL,  $t=0.68$ ,  $p=0.501$ ). The PEDF level in vitreous samples from patients with diabetes duration  $\geq 15$  years and from those with diabetes duration  $< 15$  years was 2.73 ng/mL and 3.46 ng/mL, respectively, ( $t=1.78$ ,  $p=0.079$ ) evidencing the trend toward decreased vitreous PEDF levels in patients with longer duration of the disease. Investigation of the relationship between vitreous PEDF levels and the interval from the earliest sign of proliferative changes has shown that patients whose interval was of more than 18 months had statistically significantly lower vitreous PEDF levels than patients whose interval was 18 months and less (2.73 ng/mL vs 3.57 ng/mL,  $t=2.23$ ,  $p=0.029$ ). Patients with sub-adequately controlled DM had statistically significantly lower vitreous PEDF levels than those with adequately controlled DM (2.79 ng/mL vs 3.53 ng/mL,  $t=2.07$ ,  $p=0.042$ ). No statistically significant correlation was found between the involvement of panretinal laser coagulation into treatment and vitreous PEDF levels ( $t=1.21$ ,  $p=0.28$ ).

Vitreous PEDF levels for eyes operated on for VH only, for eyes operated on for VH in the presence of macular traction detachment, and for eyes operated on for TRD/RRD were 3.75 ng/mL, 2.83 ng/mL and 2.47 ng/mL, respectively. There was no significant difference in these levels between eyes operated on for VH in the presence of macular traction detachment and eyes operated on for TRD/RRD ( $t=0.59$ ,  $p=0.56$ ). In addition, Vitreous PEDF levels were statistically significantly higher for eyes operated on for VH only than for those operated on for VH in the presence of macular traction detachment ( $t=2.35$ ,  $p=0.022$ ) as well as for eyes operated on for TRD/RRD ( $t=2.02$ ,  $p=0.049$ ).

Therefore, as proliferative changes evolve, a reduction in vitreous PEDF levels is observed, coupled with the potential for the development of retinal neurodegenerative conditions. These findings are in accordance with those of Yokoi and colleagues [14] who stated that vitreous levels of PEDF in PDR patients without VH were significantly ( $p < 0.05$ ) decreased, compared with those in PDR patients with VH. Additionally, in that study, there was no significant difference in vitreous PEDF levels between patients pre-treated with panretinal photocoagulation and those pretreated with no or focal retinal photocoagulation [14]. Abu El-Asrar and co-authors noted that (1) mean PEDF level was significantly higher in patients with active PDR undergoing vitrectomy than in patients with inactive PDR undergoing this surgery and (2) there was no significant difference in vitreous PEDF levels

**Table 1.** Parameters of photopic (cone) and scotopic (rod) ERG, mean (SD) values, in patients with PDR and below-median ( $\leq 3.12$  ng/ $\mu$ L) versus above-median vitreous PEDF levels

Vitreous PEDF level, ng/ $\mu$ L	Photopic ERG				Scotopic ERG	
	a-wave latency, ms	a-wave amplitude, $\mu$ V	b-wave latency, ms	b-wave amplitude, $\mu$ V	b-wave latency, ms	b-wave amplitude, $\mu$ V
$\leq 3.12$ , n=36	26.47 (8.50)	12.59 (9.22)	49.11 (11.48)	24.22 (8.56)	67.50 (15.33)	27.67 (20.12)
$> 3.12$ , N=34	23.85 (7.78)	15.21 (9.27)	43.85 (9.35)	29.65 (10.14)	62.68 (19.07)	34.52 (24.11)
Significance of difference	t=1.34, p=0.19	t= 1.23, =0.22	t= 2.09, p= 0.04	t= 2.43, p= 0.02	t= 1.16, p= 0.25	t= 1.29, p= 0.20

**Table 2.** Parameters of 30 Hz flicker ERG as well as of oscillatory potentials, mean (SD) values, in patients with PDR and below-median ( $\leq 3.12$  ng/ $\mu$ L) versus above-median vitreous PEDF levels

Vitreous PEDF level, ng/ $\mu$ L	30 Hz flicker ERG		Oscillatory potentials (OP)		
	P1 latency, ms	P1 amplitude, $\mu$ V	N2 latency, ms	P2 latency, ms	OP N2-P2 amplitude, $\mu$ V
$\leq 3.12$ , n=36	59.36 (15.62)	23.09 (12.66)	26.5 (7.77)	31.22 (9.41)	6.44 (2.94)
$> 3.12$ , N=34	50.97 (13.29)	30.86 (16.97)	23.09 (5.71)	27.06 (6.36)	8.19 (3.71)
Significance of difference	t= 2.41, p= 0.019	t= 2.18, p= 0.033	t= 2.08, p= 0.041	t= 2.16, p= 0.035	t= 2.19, p=0.032

**Table 3.** Parameters of mixed ERG, mean (SD) values, in patients with PDR and below-median ( $\leq 3.12$  ng/ $\mu$ L) versus above-median vitreous PEDF levels

Vitreous PEDF level, ng/ $\mu$ L	Parameters of mixed ERG				
	a-wave latency, ms	a-wave amplitude, $\mu$ V	b-wave latency, ms	b-wave amplitude, $\mu$ V	b/a ratio
$\leq 3.12$ , n=36	29.44 (6.72)	40.54 (27.91)	61.11 (11.64)	77.92 (65.22)	1.51 (0.46)
$> 3.12$ , N=34	26.65 (6.40)	52.19 (31.16)	53.53 (14.99)	106.26 (73.28)	1.69 (0.52)
Significance of difference	t= 1.78, p= 0.080	t= 1.64, p= 0.107	t= 2.35, p= 0.022	t= 1.71, p= 0.092	t= 1.57, p=0.121

between PDR patients operated on for VH only and those operated on for traction retinal detachment [4].

Tables 1 to 3 present the relationships between some electrophysiological indices of retinal neurodegeneration and vitreous PEDF levels in PDR patients subjected to intravitreal surgery.

It is seen from Table 1 that, in comparison to lower limit of normal range, there was a two-fold decrease in amplitude and latency of photopic ERG a-wave as well as in those of photopic ERG b-wave in our patients who had undergone an intravitreal surgery for complications of PDR. We observed no statistically significant difference in photopic ERG a-wave amplitude or latency between patients with above-median ( $> 3.12$  ng/mL) vitreous PEDF levels and those with below-median ( $< 3.12$  ng/mL) vitreous PEDF levels. The photopic ERG b-wave latency was statistically significantly higher and the photopic ERG b-wave amplitude was statistically significantly lower in the latter than in the former patients.

In study eyes, the amplitude of scotopic ERG b-wave was markedly (three-fold) decreased while the

latency of scotopic ERG b-wave was normal or close to normal. No statistically significant difference was found between scotopic ERG parameters and vitreous PEDF levels. This was possibly due to a marked spread in these parameters, and, correspondingly, due to an insufficient number of observations.

In this study we obtained evidence that low vitreous PEDF levels are mostly reflected in the bioelectrical activity of intermediate retinal layers (bipolar cells), and that, photopic ERG b-wave parameters may be considered as surrogate indicators of vitreous PEDF levels in patients with PDR.

In the 30-Hz flicker ERG response, the function of the cone system alone was assessed, and the latency was statistically significantly lower and the amplitude was statistically significantly higher in patients with below-median vitreous PEDF levels than in those with above-median vitreous PEDF levels. This gives evidence that, the lower is the vitreous PEDF level, the more severe is the macular dystrophic process.

The oscillatory potentials represent a series of low-amplitude high-frequency waves, and are generated

by bipolar cells (and, in a greater extent, by amacrine cells). Since they represent interrelationship of the cell elements of the inner and middle retinal layers, and are the main index of retinal ischemia, investigation of them was of special interest. In this study, the OPs demonstrated that, compared to the normal level, the latencies of positive as well as of negative peaks were markedly increased (5-fold) and the amplitudes of N2 and P2 were decreased 7-fold, evidencing that a rather marked ischemia is maintained in patients having intravitreal interventions for complications of PDR with a successful anatomic result. The OP latencies of both peaks were statistically significantly increased, while the OP amplitudes were statistically significantly decreased in patients with below-median vitreous PEDF levels compared to those in patients with above-median vitreous PEDF levels, demonstrating an anti-ischemic role of PEDF. The majority of the studies aiming to assess the OPs in patients with DM have described vitreous PEDF levels as an indicator of first signs of the ischemia in the internal retinal layers in patients without DR as well as in those with early DR [1]. As DR progresses, OP latency increases and OP amplitude decreases, and these changes are most pronounced in patients with severe PDR [3]. In this paper we assessed the OPs after intravitreal interventions (for complications of PDR) as well as the relationship of these OPs with baseline vitreous PEDF levels.

It should be emphasized that parameters of 30-Hz flicker ERG and OP may be used as surrogate indicators of vitreous PEDF levels in patients with PDR. In patients who had undergone intravitreal interventions for complications of PDR, the mixed ERG demonstrated (1) an increase in b-wave latency as well as an increase in a-wave latency, with the former being more pronounced than the latter, and (2) a 3.5-fold decrease in a-wave amplitude and a 4-fold decrease in b-wave amplitude, compared to the norm, and (3) within-the-norm mean b/a ratio.

Of note, the b-wave latency of mixed ERG was statistically significantly increased in patients with below-median vitreous PEDF levels compared to those in patients with above-median vitreous PEDF levels. Other parameters of mixed ERG did not exhibit statistically significant changes with changes in vitreous

PEDF levels. Therefore, in low vitreous PEDF levels, the most pronounced change is seen in b-wave latency of mixed ERG, thus indicating low functional activity of bipolar and Muller cells. These findings were in agreement with photopic ERG findings in our study.

In eyes operated on for complications of PDR, a statistically significant mean VA improvement was found, and the number of eyes with VA <0.09 decreased from 56 to 26 ( $\chi^2 = 28.15$ ,  $p=0.000$ ). In patients operated on for VH only, VA was statistically significantly higher compared with patients operated for MTD (0.32 (0.24) vs 0.1 (0.13),  $t=4.45$ ,  $p=0.001$ ) and with patients operated for TRD/RRD (0.32 (0.24) vs 0.12 (0.07),  $t=2.45$ ,  $p=0.030$ ).

In patients with VA of 0.01-0.09, 0.1-0.25, and 0.3-1.0, mean (SD) vitreous PEDF levels were 2.89 (1.48) ng/ $\mu$ L, 1.43 ng/ $\mu$ L (1.43) and 4.04 (1.94) ng/ $\mu$ L, respectively. The mean vitreous PEDF level was statistically significantly higher in patients with VA of 0.3-1.0 than in those with VA of 0.01-0.09 ( $t= 2.24$ ,  $p=0.030$ ). There was no statistically significant difference in vitreous PEDF levels between patients with VA of 0.3-1.0 and those with VA of 0.1-0.25 ( $t= 0.75$ ,  $p=0.457$ ) as well as between patients with VA of 0.1-0.25 and those with VA of 0.01-0.09 ( $t= 1.66$ ,  $p=0.105$ ). Therefore, in intravitreal surgeries for complications of PDR, low levels of PEDF in ocular tissues, being one of the major causes of retinal neurodegeneration, lead to statistically significantly lower functional outcomes.

In conclusion, in patients with PDR, as intraocular proliferative changes evolve and result in traction retinal detachment, vitreous PEDF levels become statistically significantly decreased. The lower was the vitreous PEDF level, the more severe was retinal ischemia, which was confirmed by electrophysiological examination (namely, the parameters of the oscillatory potentials). When the level of PEDF in the vitreous is decreased, retinal neurodegeneration develops, with the greatest damage exhibited by bipolar, amacrine, and Muller cells. Parameters of photopic and 30 Hz flicker ERG as well as of oscillatory potentials are surrogate indicators of levels of PEDF in ocular tissues, and may be used in later studies for investigation of the role of these levels in pathological processes in the eye.

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