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Serial aqueous VEGF analysis for predicting anti-VEGF treatment outcomes in neovascular glaucoma from diabetic retinopathy: a prospective analysis

Taesung Joo^{1,2} and Jin-Ho Joo^{3,4,5*}

Abstract

This study evaluated the clinical outcomes of intravitreal bevacizumab (IVB) in neovascular glaucoma (NVG) secondary to proliferative diabetic retinopathy (PDR) and investigated the association between serial aqueous vascular endothelial growth factor (VEGF) levels and treatment outcomes. A prospective single-arm observational study included 17 patients with NVG due to PDR. All patients received three bi-weekly IVB injections. Aqueous VEGF levels, intraocular pressure (IOP), and best-corrected visual acuity (BCVA) were measured before and after each injection. After the initial three injections, additional IVB injections were administered on a pro re nata (PRN) basis if IOP exceeded 21 mmHg or if iris or angle neovascularization persisted or recurred. Patients were followed for up to 3 years. After three injections, mean VEGF levels significantly decreased from 4681.46 pg/mL to 104.55 pg/mL ($P < 0.001$). BCVA improved (1.27 to 0.91 LogMAR, $P < 0.001$), and IOP decreased from (32.71 to 25.29 mmHg, $p = 0.031$). Higher baseline VEGF levels were significantly associated with worse BCVA and elevated IOP after treatment, as well as more frequent injections. Patients requiring six or more injections showed significantly higher baseline VEGF levels and poorer clinical outcomes compared to those requiring five or fewer injections. Serial aqueous VEGF levels were significantly associated with clinical outcomes and treatment burden in NVG secondary to PDR. Aqueous VEGF may serve as a potential surrogate marker reflecting disease severity and therapeutic response.

Keywords Neovascular glaucoma, Bevacizumab (Avastin®), Vascular endothelial growth factor, Aqueous humor, Diabetic retinopathy

*Correspondence:

Jin-Ho Joo

oph.jhjoo@gmail.com

¹Retina Center, Hangeil Eye Hospital, Incheon, Republic of Korea

²Department of Ophthalmology, Kyung Hee University Hospital at Gangdong, Seoul, Republic of Korea

³Department of Ophthalmology, Chung-Ang University College of Medicine, Seoul, Republic of Korea

⁴Department of Ophthalmology, Chung-Ang University Gwangmyeong Hospital, Gwangmyeong-si, Republic of Korea

⁵Institute of Clinical Medicine, Chung-Ang University Gwangmyeong Hospital, Gwangmyeong-si, Republic of Korea



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Introduction

Glaucoma is a chronic eye disease that damages the optic nerve and causes permanent blindness. The global prevalence of glaucoma has increased from 64.3 million cases in 2013 and is projected to exceed 111 million by 2040 [1]. Neovascular glaucoma (NVG) is a vision-threatening secondary glaucoma characterized by neovascularization of the iris and increased intraocular pressure (IOP). NVG accounts for 3.9% of all glaucoma cases and 9–14.7% of all secondary glaucoma, with a higher prevalence among individuals with underlying conditions causing retinal ischemia and chronic hypoxia, such as proliferative diabetic retinopathy (PDR), central retinal vein occlusion (CRVO), and ocular ischemic syndrome (OIS) [2, 3].

Retinal ischemia triggers the release of vascular endothelial growth factor (VEGF), which induces angiogenesis. In NVG, excessive VEGF enters the anterior chamber of the eye from the posterior pole, promoting the formation of pathological blood vessels in the iris and iridocorneal angle [4]. Tripathi et al. [5] demonstrated through competitive enzyme immunoassay that patients with NVG exhibit a significantly increased levels of VEGF in the aqueous humor. Similarly, Sun et al. [6] found that the concentration of aqueous VEGF-A was higher in the NVG group than that in the PDR group, with notably higher concentrations in the aqueous humor than that in the vitreous. Kim et al. [7] reported that elevated aqueous humor VEGF levels were associated with failure following Ahmed glaucoma implantation. Therefore, VEGF, an endothelial cell-specific angiogenic factor, plays a central role in iris neovascularization and NVG.

Based on these findings, anti-VEGF therapies have been employed for the treatment of ocular neovascularization. Bevacizumab (Avastin, Genentech, South San Francisco, CA), a humanized monoclonal antibody targeting all VEGF isoforms, shows promise in treating iris neovascularization and NVG [8]. The off-label use of intravitreal bevacizumab (IVB) stabilizes neovascular activity and controls IOP in patients with iris and/or iridocorneal angle neovascularization particularly in early stage NVG without angle closure, although repeated injections maybe necessary in some cases. In advanced NVG, IVB alone may not stabilize elevated IOP but can be used as a preoperative adjunct to improve the quality and safety of surgery [9]. Although these encouraging results may suggest that bevacizumab should be the first treatment of choice for NVG, the optimal dosage and injection intervals for IVB to treat NVG has not yet been established.

A few studies identified through a MEDLINE search quantitatively evaluated IVB-induced changes in VEGF concentrations in fluid from patients with advanced NVG and found that bevacizumab reduced VEGF levels in the aqueous humor [10, 11]. Sasamoto et al. showed a

significant decrease in aqueous humor VEGF levels one week after the initial injection, with a notable correlation between VEGF levels and IOP [12].

While previous studies have measured VEGF concentrations before and after treatment, these evaluations were typically conducted only once. No studies have yet serially measured VEGF levels following multiple injections. Therefore, this study investigated the effects of three consecutive bi-weekly intravitreal anti-VEGF injections in a group of patients with PDR-induced NVG. VEGF concentrations in the aqueous humor were measured and analyzed after each injection to investigate the association with IOP control and the prognosis of IVB treatment.

Methods

Subjects, method, and data analysis

This study was designed as a prospective single-arm observational study conducted at a single center. The study was approved by the Institutional Review Board of Kyung Hee University Hospital at Gangdong (KHNC 2016-12-021) and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants.

Between 2016 and 2022, patients with NVG secondary to PDR, who demonstrated uncontrolled IOP despite maximum tolerated anti-glaucomatous medications (topical β -blockers, topical carbonic anhydrase inhibitors (CAIs), topical α 2-adrenergic agonists, and systemic medications) and evidence of iris and/or angle neovascularization were included. Eligible patients were treated with three IVB injections at 2-week intervals. Patients with a history of intraocular surgery that could significantly affect aqueous VEGF levels or intraocular pharmacokinetics, such as vitrectomy or glaucoma surgery, were excluded. However, patients with a history of uncomplicated cataract surgery were not excluded, as this procedure is not expected to substantially alter aqueous VEGF levels or intravitreal pharmacokinetics. Patients who required glaucoma surgery during follow-up were withdrawn from the study at the time of surgery and were excluded from subsequent longitudinal analyses. However, data obtained prior to surgery were included in the analysis up to the time of withdrawal. No patients had undergone glaucoma surgery at baseline.

All patients received panretinal photocoagulation (PRP) to control neovascularization, particularly in cases with angle closure due to peripheral anterior synechia. However, no additional laser procedures for intraocular pressure control, such as cyclophotocoagulation or laser trabeculoplasty, were performed during the study period, except for panretinal photocoagulation (PRP), which was used solely to address retinal ischemia. Aqueous humor samples from the anterior chamber were collected before

each injection, and VEGF concentrations (pg/mL) were measured by enzyme-linked immunosorbent assay.

All patients received three consecutive intravitreal bevacizumab (IVB) injections at 2-week intervals. After the initial three injections, patients were observed for 1 month. Elevated intraocular pressure (IOP) was primarily managed with anti-glaucoma medications, including topical and systemic agents. Additional intravitreal bevacizumab (IVB) injections were administered on a pro re nata (PRN) basis when IOP remained >21 mmHg despite medical treatment or when iris or angle neovascularization persisted or recurred. Conversely, if IOP was normal or neovascularization was not present at this time, patients were followed-up two months later. At subsequent 2-month follow-up, those who required further injections were monitored 1 month after treatment, while those who did not requiring further injections were observed 3 months later. The maximum observation period was 3 months. The end point of the study was set at 3 years following the first injection. Patients were withdrawn from the study if they opted for surgery to control their IOP or declined to continue participation (Fig. 1).

Best-corrected visual acuity (BCVA, LogMAR), IOP, and VEGF concentrations before injections were analyzed using the Wilcoxon signed-rank test, and the correlation between initial VEGF concentration and BCVA or IOP after three injections was evaluated using Spearman

correlation analysis. The correlation between the initial VEGF concentration in aqueous humor and the interval to the first PRN injection after three consecutive injections, as well as the average number of PRN injections per year, was analyzed using Spearman correlation analysis. For correlation analyses, 95% confidence intervals were calculated using Fisher's z-transformation. Patients were categorized into two subgroups based on the total number of anti VEGF injections received (≤ 5 vs. ≥ 6) for exploratory analysis. This cutoff was selected based on the distribution of injection numbers within the cohort, which allowed for relatively balanced subgroup sizes. The cutoff was close to the median number of injections in the cohort. Due to the small sample size, non-parametric statistical methods were applied. Changes in BCVA, IOP, and VEGF levels before and after treatment were analyzed using the Wilcoxon signed-rank test, and correlations between baseline VEGF levels and clinical outcomes were assessed using Spearman correlation analysis. Repeated-measures approaches such as linear mixed-effects models were not applied due to the limited sample size and the potential risk of overfitting. Clinical variables including lens status, systemic comorbidities (e.g., hypertension, dyslipidemia, chronic kidney disease), and neovascular status (NVI/NVA) were collected and analyzed. All included eyes had open-angle status at baseline. Cases of NVG secondary to central retinal vein occlusion or ocular ischemic syndrome were excluded. However, detailed assessment of peripheral anterior synechiae was not consistently available.

Data were processed and statistically analyzed using SPSS for Windows (Version 27.0; SPSS, Chicago, IL). For all tests, $P < 0.05$ was considered statistically significant.

Anti-VEGF injection

All NVG patients received intravitreal injections of 0.05 mL of 1.25 mg IVB via a 30-gauge needle in the inferior temporal quadrant, positioned 3.5 mm to 4 mm posterior to the limbus. Antiglaucomatous medications and anti-inflammatory and anti-infective eye drops (prednisolone acetate ophthalmic suspension, United States Pharmacopoeia; Allergan, Inc., Irvine, CA and levofloxacin hydrate ophthalmic solution, Japan Pharmacopoeia; Santen pharmaceutical CO., LTD., Ishikawa) were administered as necessary to manage IOP and address inflammatory and/or infectious response.

Aqueous humor sampling

Aqueous humor samples were collected during each IVB treatment (pre-IVB). A 200 μ L sample of undiluted aqueous humor was obtained via anterior chamber paracentesis using a 30-gauge needle. Each aqueous humor sample was placed in an Eppendorf tube, rapidly frozen at -80°C

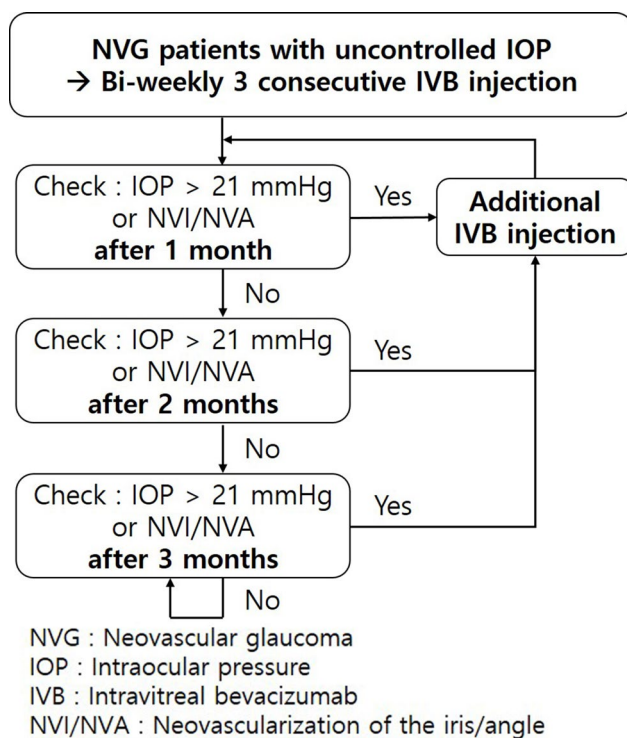


Fig. 1 Flowchart illustrating the intravitreal bevacizumab (IVB) treatment and observation in patients with neovascular glaucoma (NVG) with uncontrolled intraocular pressure (IOP)

and protected from light until the VEGF concentration was measured.

Measurement of aqueous humor VEGF concentration

VEGF concentrations in aqueous humor were measured using an enzyme-linked immunosorbent assay (Quantikine®, Human VEGF immunoassay, R&D System Inc., Minneapolis, MN). According to the protocol provided by the manufacturer. For each assay, 100 µL per sample was used. The mean of two readings was utilized for quantitative analysis. The limit of the detectable VEGF concentration was 15.6 pg/mL.

Result

Demographic data of the patients

A total of 20 NVG patients who met the inclusion criteria were included in the study. However, three patients were excluded: two eyes required glaucoma surgery due to uncontrolled IOP, and one patient voluntarily withdrew from the study. All measurements were performed successfully, with no failures resulting from insufficient sampling in VEGF determination.

Clinical parameters included BCVA, IOP, and aqueous VEGF levels. Additional clinical information such as NVI, NVA, lens status, and systemic comorbidities were also collected and analyzed. All patients had NVG secondary to proliferative diabetic retinopathy, and no cases associated with central retinal vein occlusion or ocular

ischemic syndrome were included. All eyes demonstrated open-angle status at baseline.

The mean age of patients with NVG was 63.47 ± 9.25 years, comprising 14 men and 3 women. There were 7 patients with NVI, 6 with NVA, and 4 with both NVI and NVA; there were 8 phakic eyes and 9 pseudophakic eyes. The average diabetes duration was 11.12 ± 7.13 years, and the mean HbA1c was $9.12 \pm 1.30\%$. The mean number of injection treatments received was 8.76 ± 5.77 . The anti-glaucoma medications used by the patients were topical CAI (17 patients), topical α 2-agonists (17 patients), topical β -blockers (17 patients), and systemic CAI (13 patients). All data are summarized in Table 1.

Table 2 provides detailed case-by-case data, including BCVA (LogMAR) and IOP measured before the first injection and after the third injection, as well as aqueous humor VEGF concentrations measured before the first injection and prior to the third injection. Additionally, the table includes the total number of injections, final BCVA (LogMAR), and final IOP. For patients receiving PRN injections, further details such as the interval between the first PRN injection after the initial three injections (in months), and average annual PRN injections are also provided. Clinical information regarding the patient's diabetes, NVI/NVA, lens status, and systemic comorbidities are also provided.

Table 1 Clinical demographics of the participants

Variables	Participants (n = 17)
Age mean (range), year	63.47 ± 9.25 (52–86)
Sex	
Male	14 (82%)
Female	3 (18%)
Neovascularization of the iris (NVI)	7 (41%)
Neovascularization of the angle (NVA)	6 (35%)
Both NVI & NVA	4 (24%)
Lens status	
Phakic eye	8 (47%)
Pseudophakic eye	9 (53%)
Duration of diabetes mean (range), years	11.12 ± 7.13 (0–22)
HbA1c mean (range), %	9.12 ± 1.30 (7.4–11.3)
Anti-glaucoma medications	
CAI (Carbonic Anhydrase Inhibitor)	17 (100%)
α 2-agonist	17 (100%)
β -blocker	17 (100%)
Systemic CAI	13 (76%)
Number of injections mean (range)	8.76 ± 5.77 (3–21)

Table 2 Data from 17 patients with neovascular glaucoma who participated in the study

Case	DM duration (Years)	Hb1Ac (%)	NVI/NVA	Lens status	systemic comorbidities	Initial BCVA (LogMAR)	After 3 injections BCVA (LogMAR)	Initial IOP (mmHg)	After 3 injections IOP (mmHg)	Initial VEGF (pg/mL)	Before 3rd injections VEGF (pg/mL)	Total number of injections	Interval between first PRN injection after 3 injections (months)	Average number of PRN injections per year	Final BCVA (LogMAR)	Final IOP (mmHg)
1	2	8.7	+/-	Pseudo-phakic	HTN, DL	1.10	0.30	23	11	441.2	40.5	3	-	-	0.30	11
2	12	8.4	+/+	Phakic	HTN, DL, CKD	1.40	1.40	22	25	6593.0	46.6	13	1	3.33	2.00	21
3	21	9.8	+/-	Phakic	HTN	0.70	0.70	35	20	653.5	72.5	5	6	0.67	0.52	16
4	20	11.0	+/+	Phakic	HTN	3.70	2.70	80	39	29540.0	298.0	10	1	2.33	3.70	23
5	0	9.9	+/-	Phakic	HTN	0.70	0.70	47	25	1366.0	129.6	6	3	1	0.52	19
6	20	11.3	+/+	Phakic	HTN	2.70	2.70	41	27	5477.0	150.0	8	1	1.67	2.70	25
7	10	8.1	-/+	Pseudo-phakic	HTN, CKD	0.30	0.15	15	19	220.9	62.0	5	3	0.67	0.30	20
8	9	7.6	-/+	Pseudo-phakic	HTN, CKD	1.70	0.22	13	15	99.1	52.2	4	6	0.33	0.40	12
9	16	7.4	+/-	Pseudo-phakic	-	0.70	0.40	28	22	391.6	53.0	5	3	0.67	0.30	19
10	14	7.5	-/+	Pseudo-phakic	-	1.10	1.10	35	40	824.0	157.2	21	2	6	0.52	19
11	0	9.1	-/+	Phakic	HTN	0.30	0.52	21	15	8329.0	42.1	11	1	2.67	0.30	22
12	3	9.0	-/+	Phakic	HTN	0.30	0.22	33	21	307.8	25.4	5	2	0.67	0.22	21
13	10	8.6	+/-	Pseudo-phakic	DL	0.70	0.82	16	33	268.1	130.5	18	1	5	0.82	22
14	10	11.3	-/+	Pseudo-phakic	DL	0.82	0.30	23	20	508.6	21.0	19	1	5.33	0.30	21
15	22	7.7	+/-	Pseudo-phakic	HTN	1.30	0.40	60	28	420.2	35.0	5	2	0.67	0.30	18
16	10	10.1	+/-	Pseudo-phakic	HTN	0.30	0.15	25	21	554.8	63.8	3	-	-	0.15	17
17	10	9.6	+/+	Phakic	HTN, CKD	3.70	2.70	39	49	23590.0	398.0	8	1	1.67	2.70	18
11.1		9.12				1.27	0.91	32.7	25.3	4681.5	104.5	8.76	2.00	1.92	0.95	19.1

NVI: Neovascularization of the iris, NVA: Neovascularization of the angle, HTN: Hypertension, DL: Dyslipidemia, CKD: Chronic kidney disease, BCVA: best corrected visual acuity, DM: diabetes mellitus, IOP: intraocular pressure, VEGF: vascular endothelial growth factor

Change in parameters before (pre-IVB) and after bi-weekly three consecutive IVB injections (post-IVB)

Compared to pre-IVB values, the mean BCVA (LogMAR) (1.27 ± 1.10) significantly improved after three IVB injections (0.91 ± 0.92 , $P < 0.001$). The mean IOP (32.71 ± 17.33 mmHg) significantly decreased after three IVB injections (25.29 ± 9.99 mmHg, $P = 0.031$). The mean VEGF concentration (4681.46 ± 8673.42 pg/mL) significantly decreased before the third IVB injection (104.55 ± 102.78 pg/mL, $P < 0.001$) (Fig. 2).

Correlation between initial VEGF concentration and parameters

A significant positive correlation was observed between initial VEGF concentration and post-IVB BCVA ($r = 0.737$, 95% CI: 0.397–0.899, $P < 0.001$) (Fig. 3A). Initial VEGF concentration was also positively correlated with pre-IVB IOP ($r = 0.535$, 95% CI: 0.073–0.808, $P = 0.027$) (Fig. 3B) and post-IVB IOP ($r = 0.636$, 95% CI: 0.224–0.855, $P = 0.006$) (Fig. 3C). No significant correlation was observed between initial VEGF concentration and pre-IVB BCVA ($r = 0.401$, 95% CI: -0.099–0.739, $P = 0.110$).

Correlation of PRN injections with initial VEGF concentration

In 15 out of 17 eyes, PRN injections were performed as follow-ups after three consecutive bi-weekly IVB injections. On average, the first PRN injection occurred two months after the initial three injections, with an average of 1.92 PRN injections per year. When examining these parameters in relation to the initial VEGF concentration, the interval between the first PRN injection after three consecutive injections showed a statistically significant correlation with the initial VEGF concentration ($r = -0.587$, 95% CI: -0.86 to -0.12, $P = 0.021$) (Fig. 4). Average number of PRN injections per year did not show a statistically significant correlation with initial VEGF concentration, although a P value of 0.069 was noted ($r = 0.482$).

Comparison of subgroups according to number of injections

For subgroup comparative analysis, we defined group 1 as those who received five or fewer injections ($n = 8$) and group 2 as those who received six or more injections ($n = 9$). Table 3 presents the results of a comparison of baseline clinical demographics between the two subgroups. Only baseline VEGF concentrations showed statistically significant results.

The BCVA (LogMAR) showed no statistical difference between groups at pre-IVB (0.80 ± 0.52 vs. 1.68 ± 1.33 , $P = 0.095$). However, after three injections, a statistically significant difference was noted (0.32 ± 0.18 vs. 1.44 ± 0.99 , $P = 0.010$) (Fig. 5A). The IOP showed no statistical difference between groups at pre-IVB (29.0 ± 14.7

mmHg vs. 36.0 ± 19.6 mmHg, $P = 0.416$) or after one injection (20.5 ± 5.2 mmHg vs. 26.3 ± 9.3 mmHg, $P = 0.129$), but a statistically significant difference was observed after three injections (19.6 ± 5.0 mmHg vs. 30.3 ± 10.8 mmHg, $P = 0.021$) (Fig. 5B). The VEGF concentrations showed statistically significant difference at pre-IVB (386.1 ± 177.3 pg/mL vs. 8499.5 ± 10750.8 pg/mL, $P = 0.049$), before second injection (52.2 ± 15.6 pg/mL vs. 131.4 ± 102.1 pg/mL, $P = 0.048$), and before third injections (50.5 ± 15.9 pg/mL vs. 152.5 ± 124.1 pg/mL, $P = 0.036$) (Fig. 5C).

Discussion

The results demonstrated that anti-VEGF therapy significantly reduced aqueous VEGF concentrations in patients with NVG, leading to improvements in BCVA and IOP after three consecutive bi-weekly IVB injection. However, some patients experienced poor IOP control or persistent or recurrent neovascularization of the iris and/or iridocorneal angle, requiring repeated injections. Statistically significant correlations were found between initial aqueous VEGF concentrations and BCVA, IOP, and need for anti-VEGF injections. Specifically, higher baseline VEGF concentrations were associated with worse BCVA, higher IOP, and a greater need for repeated anti-VEGF injections.

NVG is challenging to treat, often requiring the use of multiple treatment modalities simultaneously, and may require the cooperation of both retina and glaucoma specialists. According to the European Glaucoma Society, NVG management relies on two primary principles: (1) the treatment of retinal ischemia by performing PRP or intravitreal anti-VEGF injections and (2) controlling high IOP by topical and systemic pharmacological medications or surgical interventions to avoid damage to the optic nerve [13]. The most common conditions leading to NVG are ischemic eye diseases such as PDR, CRVO, and OIS, with PRP serving as the primary treatment to alleviate retinal ischemia. The goal of PRP is to reduce the ischemic areas in the retina by increasing the oxygen supply and suppressing the secretion of VEGF [14]. In addition, there are pharmacologic and surgical treatments to control IOP. Topical anti-glaucoma drugs such as topical β -blockers, α -agonists, and CAIs, may be beneficial in reducing IOP. Systemic CAIs can also be effective for temporary short-term IOP reduction but must be used carefully in patients with renal impairment [15]. However, pharmacological treatment does not provide an adequate response for elevated IOP; approximately 50% of NVG cases require surgical IOP control such as drainage valve implantation, trabeculectomy with mitomycin C or 5-fluorouracil, and cyclodestructive procedures are usually required [16].

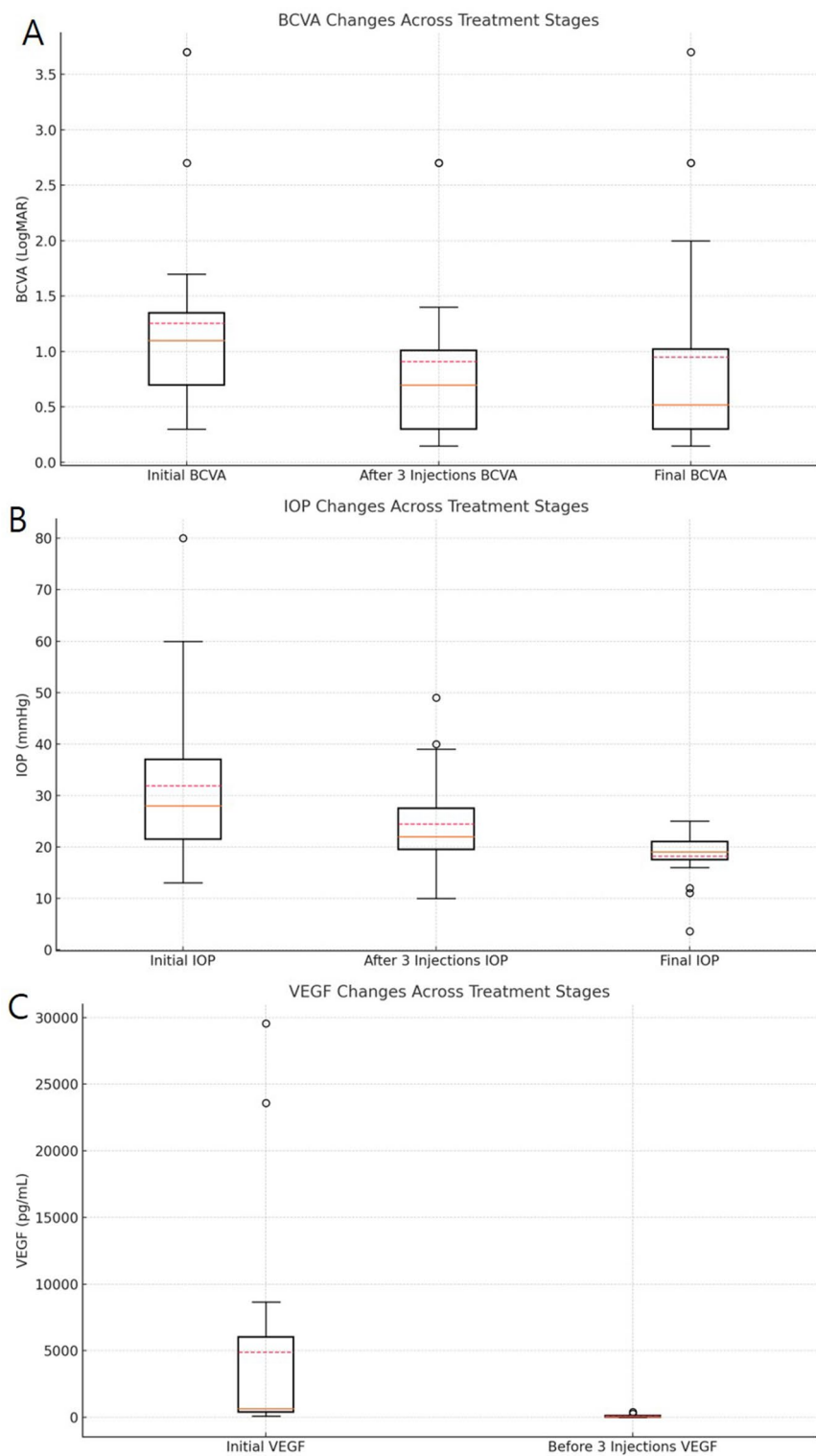


Fig. 2 Plot graphs showing changes in parameters: **(A)** best corrected visual acuity (BCVA), **(B)** intraocular pressure (IOP) at baseline, after intravitreal bevacizumab (IVB) treatment, and at the 3-year follow-up, and **(C)** vascular endothelial growth factor (VEGF) concentrations at baseline and after IVB treatment

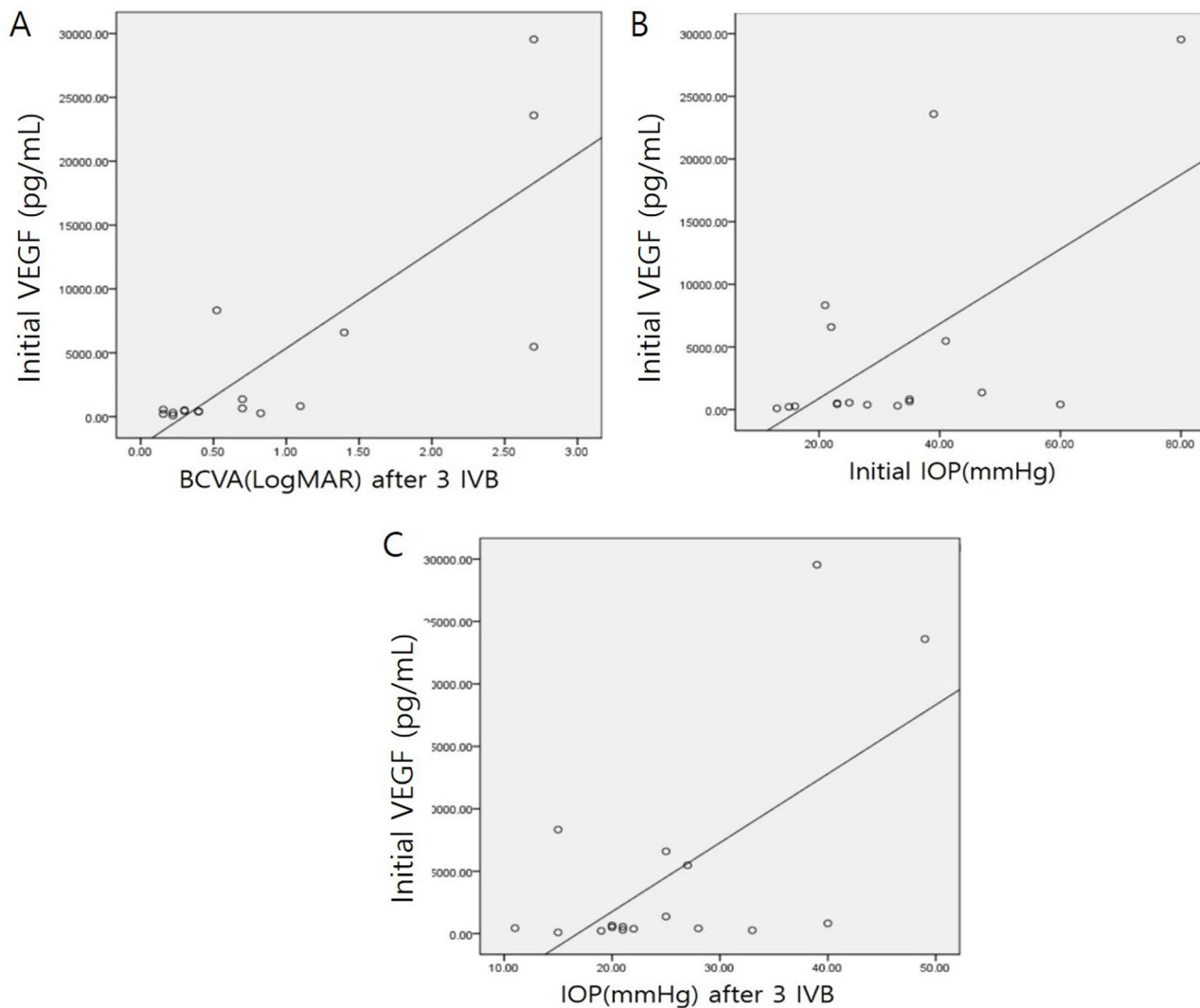


Fig. 3 Graphs showing correlations between initial vascular endothelial growth factor (VEGF) concentration and parameters: **(A)** best corrected visual acuity (BCVA) after three intravitreal bevacizumab (IVB) injection ($r=0.737$, 95% CI: 0.397–0.899, $P<0.001$), **(B)** initial intraocular pressure (IOP) ($r=0.535$, 95% CI: 0.073–0.808, $P<0.027$). **(C)** IOP after three IVB injection ($r=0.636$, 95% CI: 0.224–0.855, $P=0.006$)

Patients with NVG exhibit elevated VEGF concentrations, which can be reduced through intravitreal injection treatment of anti-VEGF inhibitors, the focus of this study. Bevacizumab, a full-length humanized recombinant monoclonal IgG antibody that inactivates all VEGF isoforms [17]. Several studies have demonstrated the efficacy of bevacizumab in NVG management. Ghanem et al. reported significant regression of iris neovascularization and IOP reduction one week after IVB [18]. Wakabayashi et al. also reported that IVB stabilizes neovascular activity and controls IOP in patients with iris and/or iridocorneal angle neovascularization alone and in early-stage NVG without angle closure [9]. Matsuyama et al. reported that IVB significantly decreased the free VEGF in the aqueous humor within seven days [11]. Additionally, Sasamoto et al. found that the rate of improved or unchanged visual acuity after IVB was higher than the

rate of worsening visual acuity [12]. Costagliola et al. reported significant improvement in visual acuity in all patients after three IVBs administered at four-week intervals [19]. While other studies have shown improvement in IOP control and neovascularization after three IVBs within four weeks, visual acuity changes were not statistically significant [20]. In clinical practice, multiple anti-VEGF agents, including bevacizumab, ranibizumab, and aflibercept, are used in the management of NVG [9, 18–22]. Available evidence suggests that these agents are effective in promoting regression of anterior segment neovascularization and short-term IOP reduction, particularly as adjuncts to PRP or surgery. However, direct head-to-head comparative evidence in NVG remains limited [14, 15]. Therefore, the relative superiority of one anti-VEGF agent over another in NVG has not yet been definitively established.

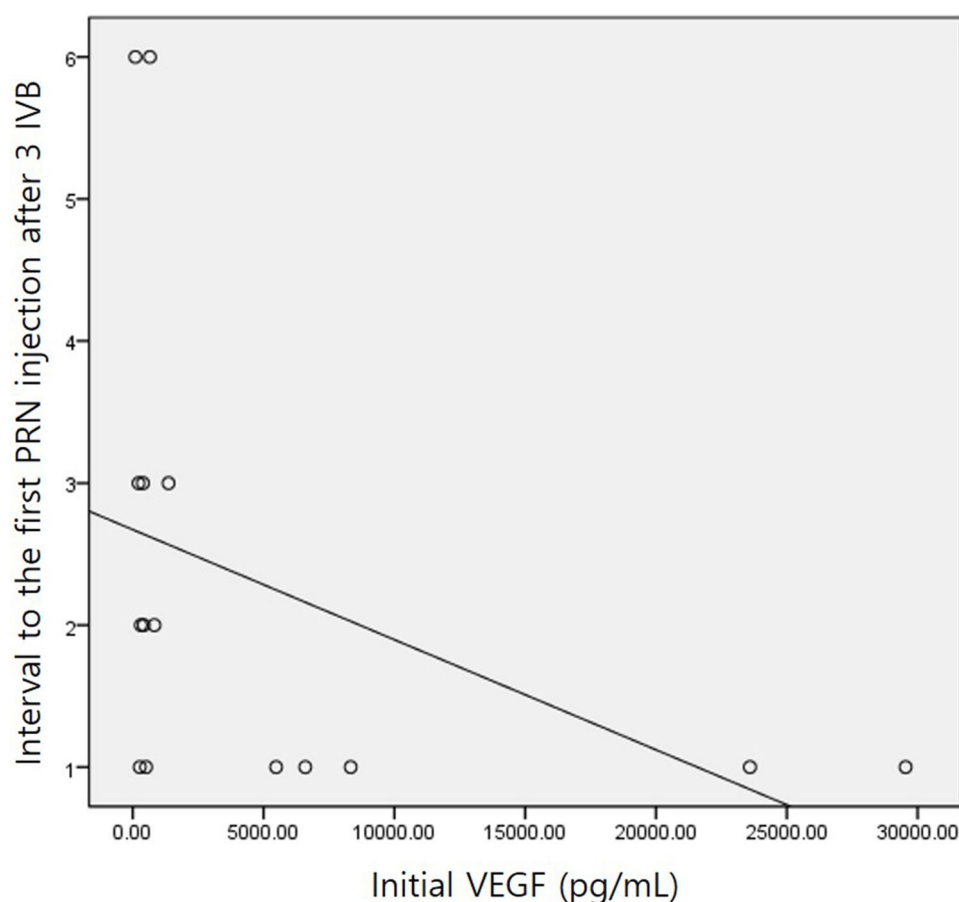


Fig. 4 Graph showing the correlation between initial vascular endothelial growth factor (VEGF) concentration and the interval to the first PRN injection following three intravitreal bevacizumab (IVB) injections ($r = -0.587$, 95% CI: -0.86 to -0.12 , $P = 0.021$)

Table 3 Baseline clinical characteristics according to the number of injections

Variable	≤ 5 injections ($n = 8$)	≥ 6 injections ($n = 9$)	P value
DM duration (years)	11.62 ± 7.50	10.67 ± 7.21	0.884
HbA1c (%)	8.6 ± 1.02	9.6 ± 1.37	0.136
Baseline BCVA (LogMAR)	0.80 ± 0.52	1.68 ± 1.33	0.157
Baseline IOP (mmHg)	29.00 ± 14.73	36.00 ± 19.63	0.470
Baseline VEGF (pg/mL)	386.14 ± 177.27	8499.5 ± 10750.79	0.006
Phakic (%)	25%	66.7%	0.153
Hypertension (%)	87.5%	66.7%	0.576

Wilcoxon signed-rank test

These findings indicate that bevacizumab shows clinical benefits in patients with NVG, suggesting it as a potential first-line treatment. However, its effects are transient due to the short half-life of the drug [12]. Several studies have investigated the pharmacokinetics of bevacizumab in rabbits and humans following intravitreal injection. In rabbits, bevacizumab reaches its peak concentration in the vitreous humor one day after injection and in the aqueous humor three days after injection, with half-lives of 4.32 and 4.88 days, respectively [23]. Another study reported peak concentration in the vitreous and aqueous humor one day after injection, with half-life of 6.61 and

6.51 days, respectively [24]. In humans, Moisseiev et al. reported a mean vitreal half-life of 4.9 days in non-vitreotomized eyes and 0.66 days in previously vitrectomized eye [25]. Csaky et al. reported that peak IVB concentrations were detected at 2–5 days post-injection, with a half-life of approximately 10 days [26]. Krohne et al. reported that concentration of bevacizumab in aqueous humor peaked on the first day after injection and a half-life was 9.82 days in non-vitreotomized eyes [27]. These findings indicate that the time to peak concentration and the half-life of bevacizumab in rabbit and human aqueous and vitreous humor are short.

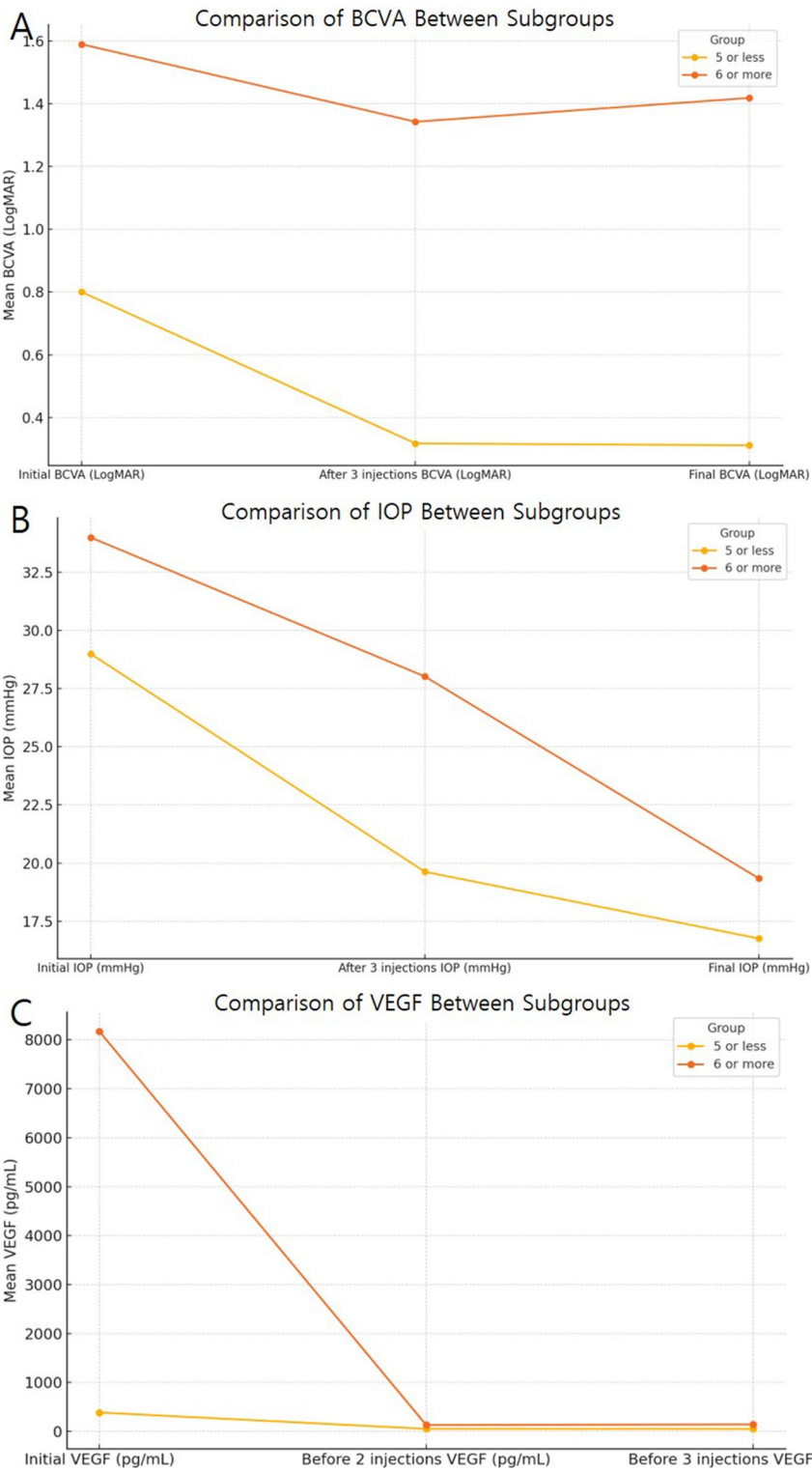


Fig. 5 Comparison of clinical parameters between subgroups based on the number of injections. Subgroups are divided into patients receiving five or fewer injections and those requiring six or more injections, with comparisons made for **(A)** best corrected visual acuity (BCVA), **(B)** intraocular pressure (IOP), and **(C)** vascular endothelial growth factor (VEGF) concentrations. BCVA and IOP were recorded before the first injection, after three injections, and at the 3-year follow-up, while VEGF levels were recorded prior to each of the three injections

This study was designed to administer three consecutive bi-weekly IVB injections in patients with PDR-induced NVG, recognizing the short intraocular half-life of bevacizumab which is less than one week. Considering the higher aqueous VEGF concentrations in patients with NVG compared to that in patients with PDR, and the elevated VEGF levels in aqueous humor relative to vitreous humor, it was hypothesized that maintaining consistent intraocular anti-VEGF levels would achieve better IOP control. In fact, several studies have shown the benefit of reducing the interval between injections in patients with refractory neovascular age-related macular degeneration (nAMD) [28]. Bi-weekly injections have been shown to significantly increase binding activity, suggesting that administration of anti-VEGF drugs every two weeks may benefit patients who respond poorly to monthly therapy [29]. In this study, NVG was considered a refractory glaucoma caused by increased VEGF levels in the anterior chamber. The results confirmed that three consecutive bi-weekly IVB injections significantly improve visual acuity, controlling IOP, and reducing aqueous VEGF concentration. A review of bi-weekly injection safety showed no issues in rabbit eyes [30]. Other studies have reported subretinal fibrosis with bi-weekly injections in patients with nAMD [31], but no retinal fibrosis was observed in the current study.

An important aspect of this study is the serial measurement of aqueous VEGF concentrations following repeated anti-VEGF injections. While previous studies have typically assessed VEGF concentrations at a single time point before and after treatment, our study provides additional insight into the dynamic changes in VEGF concentrations with repeated dosing. The marked reduction in VEGF concentrations after consecutive bi-weekly injections supports the pharmacokinetic rationale of maintaining adequate intraocular anti-VEGF activity, particularly in conditions such as NVG where VEGF concentrations are markedly elevated.

This study further established that higher initial VEGF concentration was associated with increased IOP, decreased visual acuity, a shorter interval to additional treatment, and a relatively greater number of injections required for treatment. This suggests that baseline VEGF concentrations may reflect the underlying angiogenic drive and disease severity. However, it should be emphasized that our analysis demonstrates associations rather than predictive capability, and caution is warranted when interpreting these findings in a prognostic context. While previous studies only found a correlation between VEGF concentration and IOP, they did not demonstrate associations with visual acuity or number of injections. Consistent with earlier findings, this study confirmed a positive correlation between VEGF concentration and IOP [12]. Additionally, it provided novel evidence linking VEGF

concentration to visual acuity and the frequency of IVB injections, marking a significant contribution to the field.

From a pathophysiological perspective, VEGF in NVG is primarily produced in the ischemic retina in the posterior segment and subsequently diffuses into the anterior chamber, where it contributes to iris and angle neovascularization. Therefore, aqueous VEGF concentrations measured in this study likely represent an indirect surrogate marker of posterior segment ischemic activity rather than a direct measurement of VEGF production at its source. This distinction is important when interpreting the relationship between aqueous VEGF levels and clinical outcomes.

Another notable finding is the difference in clinical outcomes between patients requiring fewer versus more frequent injections. Patients requiring six or more injections had significantly higher baseline VEGF concentrations and poorer outcomes after treatment. This further supports the concept that VEGF concentrations are associated with disease severity and treatment burden. However, the cutoff used for subgroup analysis was based on the distribution of injection numbers in this cohort and should be interpreted with caution.

This study has several limitations. First, the sample size was relatively small, which may limit statistical power and generalizability. Second, although several relevant clinical variables such as lens status, systemic comorbidities, and neovascular status were included, detailed gonioscopic findings such as the extent of peripheral anterior synechiae were not consistently available. This limitation should be considered when interpreting the results. Third, aqueous VEGF measurements may not fully capture the complexity of posterior segment VEGF dynamics. Fourth, although repeated-measures statistical approaches such as linear mixed-effects models could provide a more comprehensive analysis of longitudinal data, non-parametric methods were used due to the limited sample size. This may limit the ability to fully account for within-subject variability. Finally, based on the study design, it would have been more precise to measure VEGF concentrations by collecting fluid two weeks after the three consecutive injections. However, ethical constraints prevented the collection of aqueous humor samples following the treatment regimen. Some patients had a history of cataract surgery, which may have influenced aqueous dynamics; however, none had undergone vitrectomy or glaucoma surgery, which are known to significantly affect intraocular pharmacokinetics. This should be considered when interpreting the results.

The elevated VEGF concentrations observed in PDR-induced NVG confirm the critical role of VEGF in NVG progression and IVB administered at the initial two-week interval effectively reduces these concentrations. Furthermore, this study found that initial VEGF concentration

associated with the prognosis of visual acuity, IOP, the severity of NVG, and the number of injections required for effective treatment. Higher initial VEGF concentrations were associated with a greater number of injections and poorer visual acuity and IOP outcomes. These findings suggest that measuring VEGF concentration in the anterior chamber may serve as a potential surrogate marker reflecting disease severity. Further studies with larger cohorts are required to validate these findings and to establish their clinical applicability.

Wilcoxon signed-rank test.

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Author contributions

T.J. contributed acquisition of data, and drafting the article. J.H.J. contributed the conception and design of the study, analysis and interpretation of data, and final approval of the version to be submitted.

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Data availability

The datasets generated in the current study are available from the corresponding author upon reasonable request.

Declarations

Ethical approval

The current research followed the tenets of the Declaration of Helsinki, and all patients provided informed consent after an explanation of the study protocol. The Institutional Review Board at Kyung Hee University Hospital at Gangdong (KHNC 2016-12-021) approved this prospective study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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