Optic Nerve Head Perfusion Before and After Intravitreal Antivascular Growth Factor Injections Using Optical Coherence Tomography-based Microangiography

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Purpose: To use optical coherence tomography angiography (OCTA) to evaluate the changes in optic nerve head perfusion following intravitreal antivascular endothelial growth factor injections.

Methods: Preinjection and postinjection intraocular pressure (IOP) and OCTA images were taken of both the injected and uninjected fellow eyes.

Results: Mean preinjection IOP was 16.6 ± 4.7 mm Hg, which increased to a mean of 40.3 ± 13.0 mm Hg ($P < 0.0001$) during the first postinjection image and remained elevated at 36.1 ± 11.5 mm Hg ($P < 0.0001$) during the second postinjection image. Although no significant change was observed in flux, vessel area density, or normalized flux when comparing the OCTA preinjection and first postinjection images, a significant decrease at the second postinjection image was observed ($P = 0.03$, 0.02, and 0.03, respectively). No significant change was observed in the uninjected fellow eye during the same time period ($P = 0.47$, 0.37, and 0.38, respectively).

Conclusions: Following an antivascular endothelial growth factor injection, mean IOP increased significantly and OCTA imaging of the optic nerve demonstrated a mild but significant decrease in optic nerve head perfusion parameters. Clinicians performing these injections should be aware of these findings and monitor the status of the optic nerve in patients undergoing injections.

Key Words: antivascular endothelial growth factor, intravitreal injection, optic nerve head perfusion, optical coherence tomography angiography, intraocular pressure

(*J Glaucoma* 2019;28:188–193)

Glaucoma is characterized by chronic progressive loss of retinal ganglion cells and its pathogenesis is still poorly understood. Although many studies support the notion that mechanical damage at the level of the lamina cribrosa contributes to this loss of retinal ganglion cells, other studies have also indicated a role for vascular dysfunction at the level of the optic nerve head (ONH).[1] Prior studies using laser interferometry, laser speckle flowgraphy, laser Doppler flowmetry, and scanning laser Doppler flowmetry have reported decreased ONH perfusion in response to elevated intraocular pressure (IOP)[2–6] and others have reported improved ONH perfusion following therapeutic IOP reduction in eyes with glaucoma.[7,8] Furthermore, other studies have found that glaucomatous eyes show impaired autoregulation within the ONH compared with nonglaucomatous eyes.[9,10]

Optical coherence tomography angiography (OCTA) is a technique that can be used to noninvasively visualize the vascular function of the ONH to the capillary level.[11,12] Using OCTA, a significant decrease in ONH vascular function was observed in a rat model of elevated IOP.[13]

Millions of intravitreal antivascular endothelial growth factor (anti-VEGF) injections are administered each year to treat a number of retinal vascular disorders including neovascular age-related macular degeneration, diabetic macular edema, and retinal vein occlusion.[14–17] Typically, a standard volume of anti-VEGF medication (0.05 mL) is injected into the vitreous cavity and because of the limited compliance of the eye there is typically a rise in IOP.[18–20] Although the IOP elevation is typically transient and resolves within 30 minutes, this transient elevation is a unique opportunity to study the response of the ONH to an acutely increased IOP. The purpose of this study is to investigate the changes in the ONH perfusion before and immediately after an intravitreal injection using OCTA to determine whether the acute elevation of IOP affects ONH perfusion.

METHODS

This study followed the tenets of the Declaration of Helsinki and was conducted in compliance with the Health Insurance Portability and Accountability Act. This study was approved by the Human Subjects Division of the University of Washington (UW) and written informed consent was obtained from all subjects before imaging.

Patients who were scheduled to receive an intravitreal anti-VEGF injection were recruited from the UW Medicine Eye Institute retina clinics. Patients with a history of ophthalmic surgery other than cataract surgery were excluded. Patients with active eye infections or ocular surface disease that would preclude measurement of IOP or high-quality imaging with OCTA were also excluded.

Demographic information including patient age, sex, ethnicity, ocular and medical history, and ocular medications were collected. A baseline IOP of the eye scheduled for an injection was measured using an iCare Rebound

Received for publication August 2, 2018; accepted November 15, 2018.
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Supported in part by the American Glaucoma Society Mentoring for the Advancement of Physician Scientists (MAPS) Award, National Institutes of Health contract NEI R01-EY024158; Carl Zeiss Meditec Inc.; Research to Prevent Blindness (New York, NY). R.K.W. received research support from Carl Zeiss Meditec Inc.
Disclosure: R.K.W. has significant financial interest in the intellectual property of OCTA technology, owned by Oregon Health & Science University. The remaining authors declare no conflict of interest.
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DOI: 10.1097/IJG.0000000000001142

tonometer (Tiolat Oy, Helsinki, Finland) with the patient in an upright position, at least 30 minutes before injection. The average of 3 IOP measurements was recorded for each IOP measurement. Axial length measurements were obtained using laser interferometry (IOLMaster Model 500; Carl Zeiss Meditec Inc., Dublin, CA). Blood pressure (BP) measurements were obtained before imaging and the mean ocular perfusion pressure was calculated as (2/3)×(mean arterial pressure)−IOP, where mean arterial pressure = diastolic BP+(1/3)×(systolic BP−diastolic BP). All eyes were then scanned centered at the ONH using a 68 kHz CIRRUS HD-OCT 5000 with AngioPlex OCT Angiography system (ZEISS, Dublin, CA) (center wavelength at 840 nm) with active motion-tracking capability to minimize the effects of involuntary eye movements (Carl Zeiss Meditec Inc., Dublin, CA). Each OCTA scan cube covered a 2.4×2.4 mm^2 area. In one B-scan, there were 245 A-scan sampling points. A total of 245 transverse locations were acquired, and at each location, 4 consecutive B-scans were acquired. Baseline images of both eyes were obtained within 1 hour before injection. For each A-scan, 1024 sampling points were collected along a 2.0 mm axial scan depth. In addition, a regular optical coherence tomography (OCT) raster cube scan of the optic disc was obtained using the same prototype device to get a 3D structural data set for retinal nerve fiber layer (RNFL) thickness and ONH structural measurements. This scanning protocol collected 200×200 sampling points over a 6×6 mm^2 area centered on the optic disc.

Patients then received the scheduled intravitreal anti-VEGF injection per standard protocol. Before anti-VEGF injection, eyes were treated with topical proparacaine hydrochloride (0.5%), topical tetravisc, and topical 5% povidone-iodine solution. An eyelid speculum was placed. The intravitreal injection was then given 3.5 mm posterior to the inferotemporal limbus, in the inferotemporal quadrant through a 30-G needle. All patients were injected with 0.05 mL of medication. Immediately following the injection, the eye was irrigated with sterile eyewash with the patient seated in an upright position.

Following a single intravitreal injection, OCTA images centered at the ONH were acquired using the previously described protocol. The IOP was measured before and after each OCTA image using the iCare Rebound tonometer. The time between injection and each image acquisition was recorded. Injected eyes were imaged twice following a single intravitreal injection to assess for changes in ONH perfusion. The uninjected fellow eye was then imaged postinjection as well to serve as a control. We excluded subjects whose images were of poor quality at baseline or at the first postinjection timepoint including eyes with signal strength (SS) fell below the manufacturer recommended cutoff (SS < 6), or with significant eye movement (defined subjectively as image artifacts seen on the OCT en face images such as a horizontal frame shift larger than the average diameter of retinal vessels or a distorted oval appearance of the ONH) at baseline or at the first postinjection timepoint were excluded.

Image and Statistical Analysis

The IOP at the time of image acquisition was approximated by the average of the IOP measurements before each image acquisition. The raw data of each scan was acquired and all scans were normalized to the same SS. All normalized scans were then processed with an optical microangiography (OMAG) algorithm to extract both the structural (OCT) and blood flow (OCTA) signals. The details of the OCTA algorithm have been described previously.[11,12] Briefly, the OCTA algorithm extracted the blood flow signals by calculating the differences of the complex OCT signal between consecutive B-scan pairs; surrounding retinal tissues were considered static and thus the OCT signal remained steady over time. Because deep ONH tissue is not penetrated well with 840 nm-based OCT, the ONH tissue was further separated to focus analysis on the prelaminar tissue (between inner limiting membrane and the anterior surface of lamina cribrosa) using a semi-automated retinal layer segmentation program.[21] OCTA flow en face images were generated by detecting the flow signal with the highest intensity along the axial direction within each layer. Flux, vessel area density, and normalized flux in prelaminar tissue were measured from the OCTA flow en face images and used to quantify ONH perfusion, where flux measured the mean flow intensity within the ONH; vessel area density indicated the percentage of the ONH occupied by vessels and capillaries; and normalized flux measured the mean flow intensity within the vessels.

Statistical analysis was performed using a statistics package (GraphPad Software, La Jolla, CA). The measurements from the baseline image were compared with the measurements obtained from the first and second post-injection images using a Wilcoxon-signed rank test. Statistical significance was defined at the $P < 0.05$ level.

Assuming a mean flux of 0.32 ± 0.04 for a normal eye as previously reported,[11] a sample size of 8 eyes would provide an 80% chance to detect a 15% difference between the preinjection and postinjection flux ($\alpha = 0.05$, $\beta = 0.20$).

RESULTS

In total, 24 subjects were recruited and 18 had images of sufficient quality at both the baseline and first postinjection timepoint to be included in the analyses. The demographic information of these 18 subjects is shown in Table 1. Mean (± SD) age was 65 ± 14 years and 6 were female. Most patients (13/18) were being treated for diabetic macular edema. None had glaucoma. Mean preinjection IOP was 16.6 ± 4.7 mm Hg, which increased to a mean of 40.3 ± 13.0 mm Hg immediately postinjection ($P < 0.0001$) at 4.4 ± 1.0 minutes postinjection during the first postinjection image and remained elevated at 36.1 ± 11.5 mm Hg ($P < 0.0001$) at 6.5 ± 1.8 minutes post-injection during the second postinjection image (Fig. 1A). Mean IOP in the uninjected fellow eye also increased from preinjection levels by a small amount postinjection (17.6 ± 6.8 and 18.8 ± 7.2 mm Hg, respectively; $P = 0.04$) when measured at 7.5 ± 2.3 minutes postinjection during postinjection imaging (Fig. 1B).

No significant change was observed when comparing the OCTA preinjection and first postinjection images in the flux (0.28 ± 0.05 vs. 0.27 ± 0.07; $P = 0.28$), vessel area density (0.74 ± 0.03 vs. 0.72 ± 0.07; $P = 0.09$), or normalized flux (0.33 ± 0.05 vs. 0.31 ± 0.06; $P = 0.31$) (Fig. 1A). Thirteen subjects had images of sufficient quality at the second timepoint for analysis, where a significant decrease between preinjection and second postinjection images were observed in the flux (−0.04 ± 0.07; $P = 0.03$), vessel area density (−0.05 ± 0.08; $P = 0.02$), and normalized flux (−0.04 ± 0.06; $P = 0.03$) (Fig. 1A). Among the uninjected fellow eyes, no significant change was observed when comparing the OCTA preinjection and postinjection images in flux (0.29 ± 0.06 vs. 0.28 ± 0.07; $P = 0.47$), vessel area density (0.74 ± 0.06 vs. 0.73 ± 0.05; $P = 0.37$), or normalized flux (0.34 ± 0.05 vs.

TABLE 1. Baseline Characteristics of Subjects

Parameters	
Age (mean ± SD) (y)	65 ± 14
Range	35-92
Sex (female) (N)	6
Diagnosis (N)	
Diabetic macular edema	13
Neovascular age-related macular degeneration	4
Idiopathic CNVM	1
Blood pressure (mean ± SD) (mm Hg)	
Systolic	136 ± 21
Diastolic	79 ± 11
Ocular perfusion pressure (mean ± SD) (mm Hg)	54 ± 10
Axial length (mean ± SD) (mm)	23.7 ± 1.1
Preinjection IOP (mean ± SD) (mm Hg)	16.6 ± 4.7
No. previous injections (mean ± SD)	8.7 ± 7.6
Postinjection increase in IOP (mean ± SD) (mm Hg)	23.7 ± 12.8
Global RNFL (mean ± SD) (μm)	86.7 ± 12.4
Time after injection to first OCTA image (mean ± SD) (min)	4.4 ± 1.0
Time after injection to second OMAG image (mean ± SD) (min)	6.5 ± 1.8

CNVM indicates choroidal neovascular membrane; IOP, intraocular pressure; OCTA, optical coherence tomography angiography; OMAG, optical microangiography; RNFL, retinal nerve fiber layer.

0.32 ± 0.06; $P = 0.38$) (Fig. 1B). An example of a subject demonstrating flux, vessel area density, and normalized flux measurements in relation to the IOP in the injected eye is shown in Figure 2.

DISCUSSION

In this study, we investigated the effects of acute IOP elevation on ONH perfusion by studying eyes receiving intravitreal anti-VEGF injection using OCTA. The OCTA technique used in this study generates the blood flow signals of retinal tissue with high resolution in a noninvasive manner. Utilizing the differences in OCT signals backscattered from static retinal tissues and from open vessels with flowing blood cells, OCTA is able to extract blood flow signal by measuring the OCT signal difference acquired at the same location over time and the flow signal intensity is related to the number of red blood cells passing through the vessel cross-section, that is, flux of the blood flow.[22] We have previously demonstrated that OCTA using the OMAG algorithm is able to image retinal vessels as well as small capillaries in both the ONH and the peripapillary region with high repeatability and reproducibility compared with traditional methods.[12,23]

We found that after intravitreal anti-VEGF injection, the IOP significantly increases and decreases in flux, vessel area density, and normalized flux was observed in the injected eyes at both postinjection timepoints, which reached significance at the second timepoint. In contrast, no significant difference in any of the 3 parameters was observed in the uninjected fellow eye. This suggests that the acute elevation in IOP following an intravitreal anti-VEGF injection impairs ONH perfusion and that the impairment is unlikely to be secondary to systemic factors as the uninjected fellow eye is unaffected. In addition, our findings indicate that autoregulation of blood flow to the optic nerve appears to provide robust short-term compensation for acute IOP elevation.

The relationship between elevated IOP and decreased ONH perfusion has been reported in a number of studies.[2–4,6] In a recent study, Hashimoto et al[2] investigated the effects of elevated IOP during pars plana vitrectomy on ONH perfusion using laser speckle flowgraphy. They observed a decrease in ONH perfusion, which was maximally decreased at 5 minutes following an IOP elevation of 25 mm Hg above baseline. Riva et al[3] likewise used laser Doppler flowmetry to measure the response of the ONH blood flow and found that a rapid increase in IOP by > 100% induced a significant decrease of > 80% in ONH blood flow. In another study, using laser Doppler flowmetry, ONH blood flow decreased in most study subjects when the IOP increased to 45 or 55 mm Hg.[6]

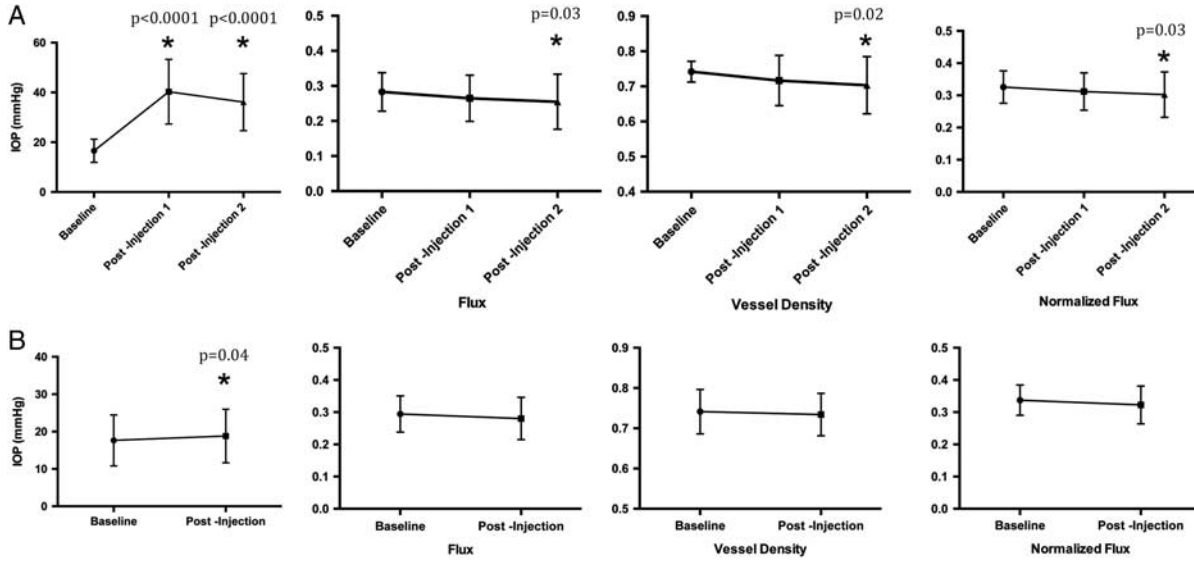

FIGURE 1. Baseline and postinjection IOP, flux, vessel area density, and normalized flux in injected (A) and uninjected (B) fellow eyes. Mean and SD shown. IOP indicates intraocular pressure. **P*-value based on Wilcoxon signed rank test of median difference equal to zero, comparing baseline and post-injection measurements.

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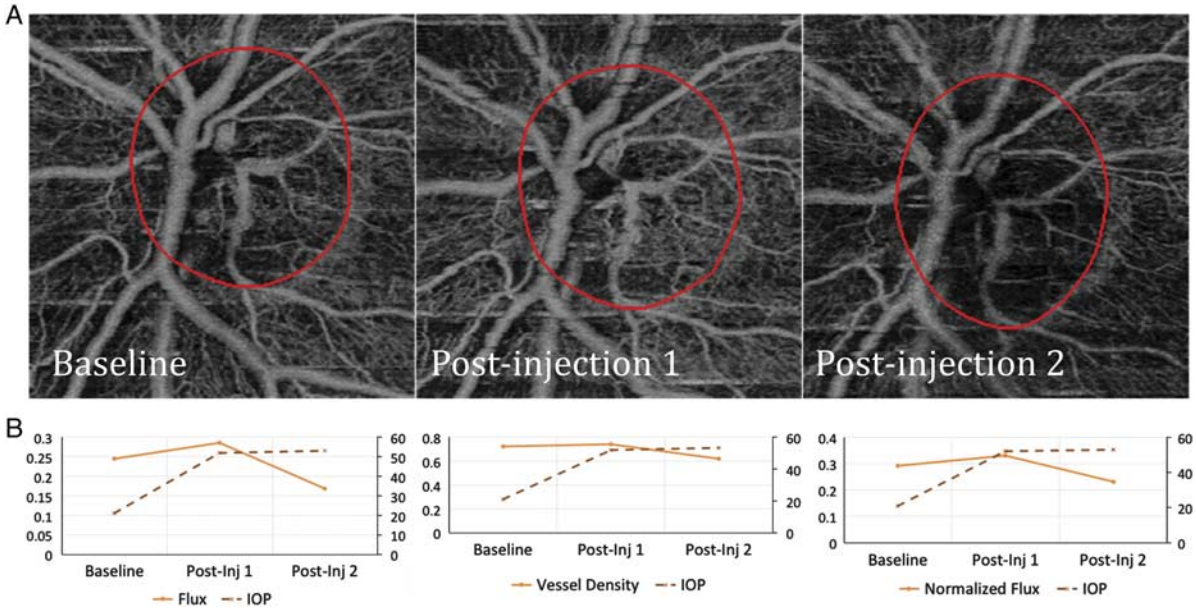

FIGURE 2. Example case of baseline, postinjection 1 and 2 optical coherence tomography angiographic images (A) and IOP, flux, vessel area density, and normalized flux (B). IOP indicates intraocular pressure. Figure 2 can be viewed in color online at www.glaucomajournal.com.

Although OCTA is a relatively new technology, it has recently been used to study the effects of IOP on the optic nerve. A study in rats using OCTA found a decrease in ONH blood perfusion following an elevation of IOP, but only once the IOP was elevated to above 60 mm Hg.[13] Zhang et al[24] studied retinal vessel density within the ONH and macula before and after IOP elevation due to a 2-hour darkroom prone provocative test and found no significant change in vessel density with IOP elevations up to 15 mm Hg above baseline. As the mean increase in IOP for our subjects was higher (23.4 mm Hg), it may be that decreases in ONH perfusion are only seen with greater IOP elevations. Conversely, lowering the IOP has been shown to increase ONH perfusion using OCTA.[25] Kim et al[25] used OCTA to image the optic nerve and peripapillary tissues before and after trabeculectomy and observed a significant increase in vessel density in the lamina cribrosa after surgery. To the best of our knowledge, our study is the first to use OCTA to demonstrate a decrease in ONH perfusion in humans following an acute IOP elevation associated with an intravitreal injection.

In most healthy optic nerves, the effects of IOP elevation on ONH blood flow and perfusion are typically tempered by autoregulation, that is, the ability to maintain a relatively constant blood flow despite changes in ocular perfusion pressure. During pars plana vitrectomy, ONH perfusion began to recover by 10 minutes after a 25 mm Hg IOP elevation.[2] In other studies, ONH perfusion and blood flow remained stable at increasing IOPs before a certain IOP threshold, also suggesting autoregulatory mechanisms at lower pressures.[4,6] Although our study identified a small but significant decrease in ONH perfusion in response to acutely elevated IOP, one limitation is that further timepoints were not obtained to determine when perfusion parameters returned to baseline and when this occurred relative to IOP. Additional research is needed to better characterize the effects of intravitreal injection on ONH perfusion parameters.

Vascular dysfunction is considered an important player in the pathogenesis and progression of glaucoma though the actual mechanism is still unclear. Numerous OCTA studies have reported decreased ONH vessel density and perfusion parameters in glaucomatous compared with normal ONHs.[11,26–28] Other studies have reported that reducing IOP significantly improved the ONH blood flow in glaucomatous eyes. Berisha et al[8] found that ONH blood flow was significantly increased in glaucomatous eyes following trabeculectomy. Hafez et al[7] found a significant improvement in ONH blood flow following therapeutic IOP reduction in patients with open-angle glaucoma but not with patients with ocular hypertension, suggesting defective autoregulation in glaucomatous eyes compared with ocular hypertensive eyes.[8] Other studies have likewise found abnormal blood flow autoregulation in eyes with glaucoma.[7–10] None of the eyes included in this study had a diagnosis of glaucoma, though these prior studies suggest that glaucomatous eyes may have even less autoregulatory mechanisms to accommodate acute elevations in IOP, and could, therefore, be more susceptible to IOP elevations associated with intravitreal injections.

It is unclear whether the acute IOP elevation and associated decrease in ONH perfusion following an intravitreal injection lead to long-term tissue changes. A number of studies have reported conflicting findings on whether intravitreal injections are associated with peripapillary RNFL thinning.[29–33] A meta-analysis by Shin and colleagues examined 6 studies on 288 eyes and concluded that there was no association between anti-VEGF injections and RNFL thickness when all studies were combined. However, when 2 studies were separately examined, there did seem to be evidence that repeated anti-VEGF injections were associated with RNFL loss. It is interesting to note that most published studies have examined eyes with a mean of ≤ 7 total intravitreal injections. Similarly, in our study subjects, the mean number of injections was 8.7 ± 7.6. Other studies

have found associations between increasing intravitreal anti-VEGF injections and sustained elevations in IOP and decreased outflow facility only after eyes had received at least 29 and 20 injections or more, respectively.[34,35] The effects of repeated intravitreal injections on RNFL thickness may not manifest until a greater number of injections have been administered but additional longitudinal studies are needed to better assess this.

Interestingly, the IOP of the uninjected fellow eye was observed to increase a small but significant amount (mean increase, 1.2 ± 2.0 mm Hg, $P = 0.04$; paired t test) following intravitreal injection. Historically, the phrase "consensual ophthalmotonic reaction" has been used to describe the phenomenon whereby changes in IOP of one eye affect the other. Studies examining this phenomenon have had conflicting findings. In a few studies, the IOP of contralateral eyes of patients who had unilateral trabeculectomy were observed to significantly increase over time.[36,37] An associated increase in aqueous production as measured by fluorophotometry in the contralateral eye following trabeculectomy has been studied as a potential mechanism for this IOP increase.[38] In contrast, a post hoc analysis of the Collaborative Initial Glaucoma Treatment Study found no significant IOP change in the contralateral eye following trabeculectomy.[39] Although most of these studies have focused on the long-term effects of IOP lowering on the contralateral eye, few have examined the acute effects of sudden IOP elevation. Older studies found that unilateral acute IOP elevation using nitrogen mustard, stimulation of cranial nerve V, and intracameral injection of prostaglandins were associated with a consensual elevation of IOP in the fellow eye.[40–42] The small but significant elevation in IOP of the uninjected fellow eye in our study subjects may represent an early consensual ophthalmotonic reaction to an acute rise in IOP following intravitreal injection.

There are several limitations to our study. First, image quality varied and so while 24 subjects were recruited, only 18 had images of sufficient quality at the first timepoint and 13 at the second timepoint to be included for analysis. This was frequently the result of ocular surface dryness or poor subject cooperation. Analysis of only the 13 subjects with images at both postinjection timepoints likewise demonstrated only a significant decrease in optic nerve perfusion at the second timepoint and not the first. Although the total number of subjects may appear low, based on previously published OCTA ONH perfusion measurements described in our methods, only 8 subjects were needed to detect a 15% change in perfusion parameters. Although a baseline BP was measured before intravitreal injection to calculate baseline mean ocular perfusion pressure, it was not logistically possible to accurately measure the BP postinjection while the patient was being imaged. Consequently, it is unknown whether changes in BP contributed to changes in the ocular perfusion pressure and therefore the observed changes in the ONH. However, given that BP changes would likely affect both eyes, one would then also expect similar decreases in ONH perfusion parameters in the uninjected eye, which was not observed. Lastly, all eyes had comorbid ocular diseases that necessitated anti-VEGF treatment. The majority of patients had diabetes, a disease with many microvascular complications, which may have affected the changes observed on OCTA. However, since many patients undergoing anti-VEGF treatment have diabetes, these findings are still very clinically relevant for this population as it suggests further ONH perfusion impairment in addition to possible baseline microvascular impairment. Future studies focusing on study subjects without diabetes, such as those with neovascular macular degeneration, are needed.

In conclusion, intravitreal anti-VEGF injections are associated with significant elevations in IOP as well as a significant decrease in ONH perfusion parameters as imaged on OCTA. Whether this leads to any long-term tissue damage remains to be seen. However, clinicians performing these injections should be aware of these findings and monitor the optic nerve in patients undergoing anti-VEGF injections.

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