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Microvasculature of the Optic Nerve Head and Peripapillary Region in Patients With Primary Open-Angle Glaucoma

Rafaella Nascimento e Silva, MD^{*,†}, Carolina A. Chiou, MD^{*}, Mengyu Wang, PhD^{*}, Haobing Wang, MS^{*}, Marissa K. Shoji, BA^{*}, Jonathan C. Chou, MD^{*}, Erica E. D'Souza, BS^{*}, Scott H. Greenstein, MD^{*}, Stacey C. Brauner, MD^{*}, Milton R. Alves, MD, PhD[†], Louis R. Pasquale, MD^{*,‡,§}, Lucy Q. Shen, MD^{*}

^{*}Department of Ophthalmology, Massachusetts Eye and Ear;

[‡]Channing Division of Network Medicine, Brigham and Women's Hospital, Boston, MA;

[§]Department of Ophthalmology, Icahn School of Medicine at Mount Sinai, New York, NY;

[†]University of Sao Paulo, Sao Paulo, Brazil.

Abstract

Purpose: To assess optic nerve head (ONH) and peripapillary microvasculature in primary open-angle glaucoma (POAG) of mild to moderate severity using swept-source optical coherence tomography angiography (OCTA).

Materials and Methods: In a cross-sectional study, swept-source OCTA images were analyzed for 1 eye from each of 30 POAG patients with glaucomatous Humphrey visual field loss and 16 controls. The anatomic boundary of ONH was manually delineated based on Bruch's membrane opening and large vessels were removed from *en face* angiography images to measure vessel density (VD) and the integrated OCTA by ratio analysis signal (IOS), suggestive of flow, in the ONH and peripapillary region. POAG subgroup analysis was performed based on a history of disc hemorrhage (DH) matched by visual field mean deviation (MD).

Results: POAG (mean MD $\pm$ SD, -3.3 ± 3.0 dB) and control groups had similar demographic characteristics and intraocular pressure on the day of imaging. Groups did not differ in superficial ONH VD or flow indicated by IOS ($P = 0.28$). POAG eyes showed significantly lower VD ($39.4\% \pm 4.0\%$) and flow ($38.8\% \pm 5.6\%$) in deep ONH, peripapillary VD ($37.9\% \pm 2.9\%$) and flow ($43.6\% \pm 4.0\%$) compared with control eyes ($44.1\% \pm 5.1\%$, $44.7\% \pm 6.9\%$, $40.7\% \pm 1.7\%$, $47.8\% \pm 2.5\%$, respectively; $P = 0.007$ for all). In the subgroup analysis, POAG eyes with ($n = 14$) and without DH ($n = 16$) had similar measured OCTA parameters ($P > 0.99$ for all).

Reprints: Lucy Q. Shen, MD, Department of Ophthalmology, Massachusetts Eye and Ear, 243 Charles St., Boston, MA 02114 (lucy_shen@meei.harvard.edu).

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Conclusions: The image processing methodology based on the anatomic boundary of ONH demonstrated compromised microvasculature in the deep ONH and peripapillary region in eyes with mild to moderate POAG, regardless of the history of DH.

Keywords

primary open-angle glaucoma; optical coherence tomography angiography; optic nerve head; disc hemorrhage

Vascular dysfunction in the optic nerve head (ONH) and the peripapillary retina is thought to be important in the pathogenesis of primary open-angle glaucoma (POAG).^{1,2} In particular, microvascular perfusion is vital for the health of the optic nerve axons.^{3,4} Alterations in the ophthalmic vasculature due to changes in perfusion pressure, vascular tone, local resistance, and even systemic dysregulation have been shown to increase the risk of glaucoma.^{5–8} In addition, pathology associated with the optic nerve vasculature, such as disc hemorrhages (DHs), can be an important indicator in the development and progression of glaucoma.^{1,9,10}

The advent of new imaging technology, specifically optical coherence tomography angiography (OCTA), has allowed for high-resolution 3-dimensional assessment of the retinal and ONH vasculature.^{11,12} This noninvasive imaging modality acquires images of the ocular vasculature in a time-dependent manner, by analyzing the motion contrast of flowing erythrocytes between consecutive stationary B scans.¹¹ Segmentation software based on corresponding optical coherence tomography (OCT) structural images provides additional details about the blood flow in the various tissue layers of the retina and ONH.^{13,14} Moreover, the detection of functional microvasculature of the ONH has been improved through novel devices such as swept-source optical coherence tomography angiography (SS-OCTA), which utilizes longer wavelengths for deeper tissue penetration, and new algorithms to process blood flow information such as OCTA by ratio analysis (OCTARA).¹⁵

Previous studies using OCTA have shown reduced vessel density (VD) and flow in the ONH and peripapillary region in glaucoma patients.^{12,16–22} However, assessment of the microvasculature within ONH has been hindered by the presence of large retinal vessels and compromised visualization of the blood supply in the laminar and retrolaminar portions of the ONH.^{12,19,22}

In this study, to overcome some of these challenges, we developed image processing methods to perform a detailed evaluation of the microvasculature in both the superficial and deep layers of the ONH as well as the peripapillary nerve fiber layer of POAG patients with mild to moderate glaucoma and normal subjects using SS-OCTA with the OCTARA algorithm. In addition, we evaluated potential differences in the microvasculature between POAG patients with and without a history of DH.

MATERIALS AND METHODS

A prospective, cross-sectional study was performed between July 2016 and April 2018 at Massachusetts Eye and Ear (MEE, Boston, MA). The research protocol was approved by the

Institutional Review Board at MEE and adhered to the tenets set forth by the Declaration of Helsinki. Informed written consent was obtained from all subjects.

Study Population

Participants of the study included adult patients aged between 35 to 82 years with POAG and age-matched control subjects from the Glaucoma Consultation Service and Comprehensive Ophthalmology Service at MEE, respectively. Many of the participants were part of previous studies conducted by our group utilizing SS-OCT,^{23,24} although all subjects were reconsented and reimaged with the new OCTA protocol described below.

Inclusion criteria for both groups were best-corrected visual acuity of 20/40 or better and refractive error within ± 6.0 D sphere and ± 3.0 D astigmatism. Exclusion criteria were significant retinal pathology (including diabetic retinopathy, age-related macular degeneration, and retinal vascular disease) and optic nerve disorders, other than glaucoma, resulting in visual field (VF) loss.

For POAG patients, additional inclusion criteria were: (1) open angles on gonioscopy, (2) glaucomatous VF loss defined as ≥ 3 contiguous points on the pattern deviation plot with ≥ -5 dB or worse for each point on at least 2 consecutive reliable Humphrey VF tests (Carl Zeiss Meditec, Dublin, CA) with fixation losses $\leq 33\%$, false-positive and false-negative rates $\leq 20\%$ on the Swedish Interactive Thresholding Algorithm (SITA) standard 24-2 algorithm,²³ and (3) glaucomatous optic neuropathy, defined as thinning or notching of the neuroretinal rim, retinal vessel changes including bayoneting, and retinal nerve fiber layer (RNFL) defects. Furthermore, glaucomatous optic nerve changes needed to show a congruent relationship with VF loss (ie, inferior optic nerve rim thinning with superior VF defect). Exclusion criteria were: (1) history of penetrating glaucoma surgery (ie, trabeculectomy or glaucoma drainage device placement, but not prior cataract surgery), and (2) significant optic disc torsion (longest axis rotation) ≥ 15 degrees outside the vertical meridian or disc tilt with the ratio between shortest and longest disc diameter <0.75 on disc photograph,^{25,26} and (3) severe glaucoma with Humphrey VF mean deviation (MD) ≤ -12 dB.

For control subjects, inclusion criteria were intraocular pressure (IOP) <22 mm Hg, cup-to-disc ratio (CDR) ≤ 0.6 in both eyes and CDR asymmetry <0.2 . Subjects with the diagnosis of glaucoma suspect or with a family history of glaucoma were excluded.

Imaging Protocol

All subjects were dilated and imaged by SS-OCT (Triton, Topcon, Tokyo, Japan) which uses a center wavelength of 1050 nm and a scanning speed of 100,000 A-scans per second.¹⁵ OCT angiography along with simultaneous structural imaging was obtained over 3×3 mm and 4.5×4.5 mm fields centered automatically on the ONH by the alignment guidance circles provided by the SS-OCT machine (Supplementary Fig. 1, Supplemental Digital Content 1, <http://links.lww.com/IJG/A228>). The SS-OCT device detected blood vessels including microvasculature through a processing method called OCTARA.¹⁵

For structural measurements, our SS-OCT imaging protocol included a radial scanning pattern with 12 sequential images and a 5-line cross-scanning pattern with 5 horizontal and 5

vertical lines spaced 250 μm apart, all centered in the ONH. Each image had 16 to 32 frames averaged. Measurement of the horizontal lamina cribrosa depth (LCD) was performed by 2 independent readers (R.N.S., L.Q.S.) using a previously published method.²⁴ Briefly, 1 horizontal scan from the 5-line cross-pattern or 1 radial scan was selected for best centration and visualization of the anterior lamina cribrosa (LC). LCD was the average distance between a reference line connecting the Bruch's membrane (BM) opening and the anterior LC surface calculated with a customized plugin in ImageJ (ImageJ; US National Institutes of Health, Bethesda, MD).²⁷ At the same visit, peripapillary RNFL was imaged with spectral-domain OCT (SD-OCT; Spectralis, Heidelberg Engineering GmbH, Heidelberg, Germany). Average peripapillary RNFL thickness was obtained automatically from SD-OCT scans.

One eye per subject was included. Eyes were excluded if the OCTA image quality score was <48 according to the OCT manufacturer, if significant motion artifacts were present or if the automated segmentation software failed to detect specified retinal layers described below. If both eyes were eligible, the eye with less depressed MD in Humphrey VF was selected in the POAG group and 1 eye was randomly selected in each control subject. Subgroup analysis was performed in the POAG group based on a history of DH documented in the clinical records and/or in a disc photograph. The POAG eyes with and without a history of DH were matched by VF MD.

Angiography Image Processing Protocol

For the quantitative analysis of the microvasculature within the ONH, the *en face* OCTA image from the 3×3 mm scan was used. An example is shown in Figure 1 from the right eye of a 65-year-old patient with POAG and DH at the time of imaging (Fig. 1A), Humphrey VF demonstrating inferior loss (Fig. 1B) with MD of -8.69 dB and thinning of the superior and nasal RNFL (Fig. 1C). With the aid of the viewing software (IMAGEnet 6) provided by the OCTA device, which enables linking of structural OCT scans to angiograms and uses an automated segmentation software to select retinal layers based on structural imaging, the superficial angiogram of the ONH was generated from the internal limiting membrane (ILM) to BM and the deep angiogram from BM to 390 μm below (Figs. 1D, E, G, H and Supplementary Fig. 2, Supplemental Digital Content 1, <http://links.lww.com/IJG/A228>). For the peripapillary microvasculature, an angiogram was generated from the ILM to the interface of RNFL and ganglion cell layer from the 4.5×4.5 mm scans (Figs. 1F, I).^{20,28}

All structural OCT scans (320 horizontal scans per eye) along with the superficial and deep angiograms were imported into ImageJ Fiji software and analyzed by 2 independent readers (R.N.S., C.A.C.) masked to diagnosis. The ONH margin was defined as the termination of BM/retinal pigment epithelium (BM/RPE).²⁹ Using a customized ImageJ plugin, termination of the BM/RPE complex was marked manually on OCT cross-sectional images spaced 47 μm apart covering the entire ONH (Figs. 1G, H and Supplementary Fig. 3, Supplemental Digital Content 1, <http://links.lww.com/IJG/A228>). The outline was then superimposed on the corresponding OCTA images to delineate the ONH margin (Figs. 1D, E and Supplementary Fig. 3, Supplemental Digital Content 1, <http://links.lww.com/IJG/A228>). The ONH area was considered to be the area inside of the margin. The peripapillary region

was defined as the 0.70-mm-wide elliptical annulus extending from the optic disc margin (Fig. 1F).¹⁶

The area occupied by large vessels was excluded from OCTA images of the ONH by manual selection in the *en face* superficial ONH angiogram with the aid of filtering images to blur smaller vascular structures (Fig. 1J). To avoid projection artifact, the same area was excluded on the deep angiogram (Fig. 1K). In the peripapillary region, the large vessels were automatically excluded using customized software in ImageJ Fiji program (Fig. 1L).

After removal of large vessels, VD was measured on *en face* OCTA images. Binary images were generated with a customized thresholding method optimized to select microvasculature. VD was defined as the percentage area occupied by the microvasculature within the BM opening of ONH or the peripapillary region after exclusion of the area occupied by large blood vessels (Supplementary Fig. 4, Supplemental Digital Content 1, <http://links.lww.com/IJG/A228>). The signal intensities of the blood vessels in *en face* OCTA images represent the scaled relative OCTARA values, which are similar to amplitude decorrelation measurements and correlates with the flow; that is, vessels with more flow in volume or speed would show up as a brighter signal (direct communication with Charles Reisman, Topcon). The surrogate quantitative flow parameter, known as the integrated OCTA by ratio analysis signal (IOS) in our study, is the mean grayscale value of the *en face* OCTA image in the microvasculature area selected with a thresholding method normalized by the constant maximum pixel intensity and shown as a percentage.

Statistical Analysis

Data analysis was performed using R Language Platform (Version 3.4.3; R Foundation, Vienna Austria). The 2-tailed student t test was used to test group differences for continuous variables. The Fisher exact test was used to compare frequencies of categorical variables between groups. For subgroup analysis, 1-way analysis of variance (ANOVA) was performed to compare continuous variables between 3 groups with Bonferroni correction for multiple comparisons. Interreader reproducibility was assessed using the intraclass correlation coefficients (ICC). $P < 0.05$ was considered significant.

RESULTS

A total of 59 subjects met the study's inclusion criteria and underwent SS-OCTA imaging. Of those, 13 subjects (22.0%) were excluded due to poor quality OCTA images. Therefore, 46 eyes from 46 subjects (30 POAG and 16 controls) were included in the analysis. There were no significant differences between the POAG and control groups in sex (overall 45.7% male), age (average 63.3 ± 8.6 y), ethnicity (93.5% Caucasian), visual acuity [logarithm of the minimum angle of resolution (LogMAR) 0.07 ± 0.10] and IOP on the day of imaging (14.0 ± 2.8 mm Hg; $P > 0.07$ for all, Table 1). The POAG patients treated with topical ocular hypotensive medications at the time of imaging as mono-therapy or as combination therapy were as follows: 63.3% of eyes were on prostaglandin analogs, 63.3% were on β blockers, 50.0% were on carbonic anhydrase inhibitors, and 36.7% were on α agonists. For the POAG patients, the eye with less severe Humphrey VF MD was selected and the average MD and pattern standard deviation (PSD) were -3.3 ± 3.0 dB and 4.4 ± 3.3 dB, respectively. POAG

eyes had significantly lower average RNFL thickness compared with controls (71.3 ± 14.1 vs. $96.5 \pm 8.9 \mu\text{m}$; $P < 0.001$), but did not differ significantly in horizontal LCD (393.5 ± 86.1 vs. $443.6 \pm 109.9 \mu\text{m}$; $P = 0.10$).

All OCTA parameters had good interreader agreement (ICCs = 0.982, Supplementary Table A, Supplemental Digital Content 2, <http://links.lww.com/IJG/A229>). Analysis of OCTA images of the ONH showed similar VD and IOS measurements in the superficial microvasculature between POAG eyes and control eyes, but a significant decrease in both angiographic parameters of the deep microvasculature in POAG eyes compared with control eyes (Table 2). There was significantly lower VD in the deep ONH of POAG eyes ($39.4\% \pm 4.0\%$, Fig. 1) than in control eyes ($44.1\% \pm 5.1\%$, $P = 0.003$, Supplementary Fig. 5, Supplemental Digital Content 1, <http://links.lww.com/IJG/A228>); this difference was not present if images were analyzed with large vessels and their projection artifacts were included (Supplementary Table B, Supplemental Digital Content 2 <http://links.lww.com/IJG/A229>; Supplementary Fig. 4, Supplemental Digital Content 1, <http://links.lww.com/IJG/A228>). IOS was also significantly lower in the deep ONH of POAG eyes ($38.8\% \pm 5.6\%$) compared with control eyes ($44.7\% \pm 6.9\%$; $P = 0.007$). In the peripapillary region, both VD and IOS were reduced in POAG eyes ($37.9\% \pm 2.9\%$ and $43.6\% \pm 4.0\%$, respectively) relative to control eyes ($40.7\% \pm 1.7\%$ and $47.8\% \pm 2.5\%$, respectively; $P < 0.001$ for both).

Subgroup analysis of the POAG eyes was performed based on a history of DH. The mean time from the documented DH to OCTA imaging was 3.2 ± 2.8 years. All DHs were noted to be located in the inferior or inferotemporal quadrant of the ONH. The POAG subgroups with and without DHs did not differ in MD, PSD, RNFL thickness, horizontal LCD, and number of glaucoma medications ($P = 0.53$ for all, Table 3). Both POAG subgroups (with and without DH) had reduced VD in the deep ONH layer compared with controls ($39.1\% \pm 4.3\%$ vs. $41.1\% \pm 5.1\%$ and $39.3\% \pm 4.5\%$ vs. $41.1\% \pm 5.1\%$, respectively; $P = 0.02$ for both). A significant difference in the IOS of the deep ONH layer was found between POAG eyes without DH and controls ($38.4\% \pm 5.6\%$ vs. $44.7\% \pm 6.9\%$; $P = 0.02$), but not between POAG eyes with DH ($39.2\% \pm 6.2\%$; $P = 0.09$) and controls. The 3 groups did not differ in superficial ONH OCTA parameters ($P > 0.99$ for all). A significant decrease in VD in the peripapillary region was found between POAG with DH ($37.6\% \pm 2.2\%$) and controls ($40.7\% \pm 1.7\%$; $P < 0.001$), but not between POAG without DH ($38.4\% \pm 3.6\%$; $P = 0.09$) and controls. Both POAG subgroups (with and without DH) showed reduced IOS in the peripapillary region compared with controls ($44.0\% \pm 2.6\%$ vs. $47.8\% \pm 2.5\%$, $P = 0.001$; $43.7\% \pm 5.0\%$ vs. $47.8\% \pm 2.5\%$, $P = 0.02$, respectively). Interestingly, comparing POAG eyes with and without DH did not yield any significant differences in all of the ONH and peripapillary OCTA parameters ($P > 0.99$). As all DHs occurred in the inferior half of the ONH in our study, we compared OCTA parameters of the inferior 180 degrees of the ONH and peripapillary region but did not find significant differences between the POAG subgroups ($P = 0.28$, Supplementary Table C, Supplemental Digital Content 2, <http://links.lww.com/IJG/A229>).

In 1 POAG patient, a DH was present at the time of OCTA imaging (Fig. 1). Individual analysis of this case showed that the ONH area had a greater superficial VD (46.8%) and IOS (50.4%), but lower deep VD (36.5%), and IOS (35.5%) compared with the mean ONH

superficial VD ($41.8\% \pm 3.8\%$), superficial IOS ($39.9\% \pm 5.0\%$), deep VD ($40.9\% \pm 5.1\%$), and deep IOS ($40.8\% \pm 6.7\%$) of the entire cohort (POAG and controls). This patient also showed lower VD (33.1%) and IOS (42.7%) in the peripapillary region compared with the averages of the cohort ($39.0\% \pm 2.9\%$ and $45.2\% \pm 4.0\%$, respectively). An additional subgroup analysis stratifying the POAG patients based on maximum IOP did not find significant differences in OCTA parameters between normal tension glaucoma (NTG, IOP 21 mm Hg) and high tension glaucoma (HTG, IOP > 21 mm Hg). When compared with controls, HTG and NTG patients both had decreased peripapillary VD and IOS, and NTG patients also showed decreased VD and IOS in the deep ONH layer (Supplementary Table D, Supplemental Digital Content 2, <http://links.lww.com/IJG/A229>).

DISCUSSION

In this study, we utilized SS-OCTA to perform a detailed analysis of the microvasculature in the ONH and peripapillary region in POAG eyes with mild to moderate severity and control eyes. The results suggest that POAG eyes have compromised blood flow and decreased microvasculature in the deep ONH and the superficial peripapillary regions compared with normal eyes regardless of the history of DH. Utilization of SS-OCTA and the OCTARA algorithm enabled visualization of the microvasculature in both the superficial and deep layers of the ONH with high resolution and reproducibility. Furthermore, the methodology we used for image processing delineated the anatomic boundary of the ONH and removed the high signal intensity associated with large blood vessels to improve the quantification of the microvasculature in the ONH and peripapillary region.¹⁹ We believe that the meticulous methodology used in this *in vivo* quantitative assessment will help to standardize future OCTA research on ONH microvasculature and perfusion.

In the present study, we focused on the ONH region as it has been well established that microvascular compromise leads to ganglion cell damage in POAG.^{3,30,31} The ONH, which is divided into the superficial nerve fiber layer (SNFL), prelaminar, laminar, and retrolaminar layers, is primarily supplied by branches of the short posterior ciliary arteries and the central retinal artery, although the exact anatomic contributions remain an area of investigation. In general, it is thought that the SNFL is mainly supplied by the branches of the central retinal artery, while the prelaminar, laminar, and retrolaminar layers are primarily supplied by branches of the short posterior ciliary arteries.^{31,32} Our study attempted to isolate the superficial and deep layers of the ONH based on vascular supply. As the currently available segmentation software in SS-OCTA does not automatically detect the LC, we generated superficial and deep layers of the ONH using BM as a landmark. The superficial layer, defined as the angiogram from the ILM to BM, was sufficient to include the full thickness of the SNFL and prelaminar tissue. The deep layer, defined as the angiogram from BM to 390 μm below, was sufficient to include the laminar region. The localization of the superficial and deep layers of the optic nerve anatomy was confirmed by manually reviewing segmentation images (Figs. 1G, H) for all eyes to ensure that the layers were selected appropriately. Eyes were excluded if the automated segmentation failed in detecting the specified layers. Segmentation based on individual anatomic structures (ILM and BM) rather than a predefined thickness for the superficial layer allowed us to measure the microvasculature of the same tissue layers despite differences in tissue layer thickness of the

ONH in POAG patients compared with controls. This is particularly important as prelaminar and laminar thickness has been found to be lower in POAG patients, although the segmentation software did not allow for adjustment for laminar thickness.^{33,34} In addition, excluding superficial large vessels on the deep angiogram allowed us to eliminate the associated projection artifacts (Fig. 1E) and improve quantification of the deep microvasculature (Supplementary Fig. 4, Supplemental Digital Content 1, <http://links.lww.com/IJG/A229>).^{22,35}

Although previous research describes generalized compromise in the vasculature of the ONH and surrounding regions,^{12,16–22} our results show a significant decrease in VD and blood flow in the deep layer of the ONH in POAG eyes compared with control eyes. These findings are consistent with the current literature that implicates the posterior lamina as the primary site of disruption in glaucoma. For instance, a histologic study of the ONH in human eyes with glaucoma found that the scleral LC was the site of damage.³ Furthermore, a study utilizing a mathematical model suggested that the central area of the LC was more vulnerable to reduced blood supply following IOP elevation.³⁶ In addition, Kang et al⁴ found that microvascular density correlates with retinal ganglion cell axonal volume in all regions of the ONH, but at a particularly greater ratio in the posterior LC, emphasizing the importance of this region for studies involving vascular parameters.

Our results also suggest that the POAG group had significantly lower VD and blood flow indicated by IOS in the superficial peripapillary layer compared with control eyes. This is consistent with previous OCTA studies using similar segmentation slab for the superficial peripapillary layer, although these studies utilized SD-OCTA devices instead of SS-OCTA and a different image processing algorithm.^{20,28}

In contrast to 2 previous reports,^{17,21} we did not find a significant difference in the superficial microvasculature of the ONH in POAG eyes compared with controls. Akil and colleagues used the same device utilized in our study to show decreased VD in the superficial layer of ONH in POAG patients, but defined the superficial layer slightly differently (ILM to RPE rather than ILM to BM) and did not exclude large vessels nor defined the ONH boundary based on structural landmarks.¹⁷ Bojikian et al²¹ used SD-OCTA with a different processing algorithm (optical micro-angiography) to isolate the blood vessels in the prelaminar layer and found reduced VD and flow indicated by flux in glaucomatous eyes. Although the margin of ONH was based on BM opening, Bojikian and colleagues also did not exclude large vessels. The difference in results between studies highlights the importance of standardizing methodology for analyzing OCTA images. We believe that ONH boundary delineation and exclusion of large blood vessels are critical to assessing the microvasculature of the ONH, as the difference in VD in the deep ONH layer was no longer apparent if large vessels and their associated projection artifacts were included in the image analysis (Supplementary Table B, Supplemental Digital Content 2, <http://links.lww.com/IJG/A229>; Supplementary Fig. 4, Supplemental Digital Content 1, <http://links.lww.com/IJG/A228>).

Our subgroup analysis compared POAG eyes with and without a history of DH and did not find significant differences in OCTA parameters. These findings are consistent with a recent

study that utilized SD-OCTA to compare VD in the ONH (in a slab from 2000 μm above to 150 μm below the ILM) and peripapillary region (defined as a 0.75 mm wide elliptical annulus from the optic disc boundary), which found no significant difference between POAG eyes with and without DH.³⁷ However, these findings differed from another study utilizing SS-OCTA, which showed more frequent microvascular dropout in the choroidal layer of the peripapillary region in Korean patients with glaucoma and DH, although quantifications of VD and blood flow were not provided.³⁸ We also separately analyzed the vasculature of the inferior half of the ONH and peripapillary region, which anatomically corresponded to the location of all the DHs in our study, but again did not find any significant differences. If vascular dysregulation is implicated in the pathophysiology of glaucoma, and DH is not found to have an independent association, one could propose that DH is not a discrete event but rather a part of the continuum of vascular dysfunction. In other words, there is vascular dysfunction in POAG eyes, regardless of the patient's history of DH. This was further supported by our second subgroup analysis, which did not find significant differences in OCTA parameters between HTG and NTG patients in the study. Other explanations for our OCTA findings on patients with DH include the occurrence of undetected DH in the POAG group without DH in the interim between their clinic visits,³⁹ and compensatory changes in the vasculature accompanying a DH. This is supported by the analysis of an eye with a DH present at the time of imaging (Fig. 1), which showed higher superficial VD and flow but lower deep VD and flow of the ONH compared with the mean of the entire cohort. Hence, it is possible that DH may result in compensatory mechanisms to increase blood flow in the superficial layer of the ONH, while the compromise in blood flow in the deep layer remains unaffected. However, the long duration between documented DHs and imaging dates in our study (average of 3.2 y) does not allow for a more detailed analysis of potential short-term effects of DHs.

This study has several limitations. First, the generalizability of our study may be limited as this study has a relatively small sample size, consists predominantly of Caucasians, and excludes eyes with high myopia, tilted discs, or advanced glaucoma. Second, the design of the study does not allow for exclusion of the effect of ocular hypotensive medications and systemic blood pressure medications on the optic nerve microvasculature. Third, the presence and extent of peripapillary atrophy was not analyzed and may also affect the ONH vascular supply, although we excluded patients with significant peripapillary atrophy resulting in VF loss.⁴⁰ Fourth, the high percentage of patient exclusion based on OCTA image quality (22%) indicates that this imaging technology still needs improvement. Fifth, projection artifacts may affect the evaluation of microvasculature in the deep layers, even with the removal of large superficial blood vessels.³⁵ Sixth, blood flow measurements were based on a surrogate indicator, as they were indirectly measured by IOS, the raw angiographic pixels within the vasculature. The exact relationship between IOS and blood flow has not been established and requires further investigation. Finally, the cross-sectional nature of this study does not allow for conclusions regarding the sequential relationships between blood flow and functional loss in POAG or between blood flow and the development of DH.

In conclusion, we utilized SS-OCTA and a quantitative methodology, which is based on the anatomic boundary of ONH and isolates the microvasculature, to evaluate the optic nerve

vasculature in patients with POAG of mild to moderate severity. Our findings suggest that there is compromised blood flow and decreased microvasculature in the deep ONH and the superficial peripapillary regions in POAG eyes compared with normal eyes. These findings support the association between vascular pathology and POAG and provide more detailed localization of this pathology within the ONH of POAG patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1.

OCT angiography analysis. Optic disc photograph of a right eye with primary open-angle glaucoma and an inferotemporal disc hemorrhage (A), its corresponding visual field pattern deviation plot (B) and peripapillary RNFL thickness profile (C). The 3×3 mm *en face* swept-source OCT angiograms were generated for the superficial layer (D) and deep layer (E) of the ONH, with circles indicating the disc margin generated by manual identification of BM opening. The BM opening (dots) was marked in the set of cross-sectional OCT scans obtained during imaging (G, H). The superficial layer of the ONH extends from the ILM to BM (G, dark band), while the deep layer extends from BM to 390 μm below (H, light band). Areas occupied by large vessels were excluded semiautomatically from the superficial layer and superimposed on the deep layer to limit projection artifact. The regions of the ONH analyzed after removal of large blood vessels are shown for the superficial layer (J) and deep layer (K). The 4.5×4.5 mm angiogram of the superficial retinal layer (F) was used in the peripapillary analysis, with lines indicating a 0.70 mm wide elliptical annulus extending from the disc margin generated from manually marking the BM opening on cross-sectional OCT scans. The superficial retinal layer (I, band) extends from the ILM to the interface of RNFL and ganglion cell layer. Large vessel exclusion was performed automatically, and the peripapillary region analyzed after removal of large vessels is shown (L). BM indicates Bruch's membrane; ILM, internal limiting membrane; OCT, optical coherence tomography;

ONH, optic nerve head; RNFL, retinal nerve fiber layer. Figure 1 can be viewed in color online at www.glaucomajournal.com.

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TABLE 1.**Baseline Characteristics of Study Population**

Systemic and Ocular Characteristics	Control (N = 16)	POAG (N = 30)	P
Subjects			
Sex (male) (%)	50.0	43.3	0.76
Age (y)	67.9 ± 8.8	65.4 ± 8.5	0.37
Ethnicity, Caucasian (%)	93.8	93.3	> 0.99
Systemic disease (%)			
Diabetes mellitus	31.3	10.0	0.11
Systemic hypertension	43.8	33.3	0.53
Hypertensive medication usage	43.8	40.0	> 0.99
Ophthalmologic findings			
Visual acuity (LogMAR)	0.04 ± 0.05	0.08 ± 0.11	0.07
IOP at the imaging visit (mm Hg)	14.4 ± 2.5	13.8 ± 3.0	0.45
Humphrey visual field			
MD (dB)	NA	-3.3 ± 3.0	NA
PSD (dB)	NA	4.4 ± 3.3	NA
Glaucoma medications (% of eyes)			
Prostaglandin analogs	0.0	63.3	NA
β blockers	0.0	63.3	NA
Carbonic anhydrase inhibitors	0.0	50.0	NA
α agonists	0.0	36.7	NA
ONH parameters			
Average RNFL (μm)	96.5 ± 8.9	71.3 ± 14.1	< 0.001 *
Horizontal LCD (μm)	393.5 ± 86.1	443.6 ± 109.9	0.10

Data are expressed as mean ± SD unless otherwise specified.

* Statistically significant difference, $P < 0.05$.

IOP indicates intraocular pressure; LCD, lamina cribrosa depth; Log-MAR, logarithm of the minimum angle of resolution; MD, mean deviation; NA, not available; ONH, optic nerve head; POAG, primary open-angle glaucoma; PSD, pattern standard deviation; RNFL, retinal nerve fiber layer.

TABLE 2.

Comparison of OCT Angiography Parameters

OCT Angiography Parameters	Control (N = 16)	POAG (N = 30)	P
ONH VD (%)			
Superficial vasculature	41.8 ± 3.2	41.7 ± 4.4	0.93
Deep vasculature	44.1 ± 5.1	39.4 ± 4.0	0.003 [*]
ONH IOS (%)			
Superficial vasculature	38.5 ± 3.9	40.0 ± 5.3	0.28
Deep vasculature	44.7 ± 6.9	38.8 ± 5.6	0.007 [*]
Peripapillary VD (%)	40.7 ± 1.7	37.9 ± 2.9	< 0.001 [*]
Peripapillary IOS (%)	47.8 ± 2.5	43.6 ± 4.0	< 0.001 [*]

Data are expressed as mean ± SD unless otherwise specified.

^{*} Statistically significant difference, $P < 0.05$.

Superficial vasculature is defined as the vessels from internal limiting membrane to BM. Deep vasculature is defined as vessels from BM to 390 µm below.

BM indicates Bruch's membrane; IOS, integrated OCT angiography by ratio analysis signal; OCT, optical coherence tomography; ONH, optic nerve head; POAG, primary open-angle glaucoma; VD, vessel density.

TABLE 3.

Comparison Between Control, POAG Without DH and POAG With DH Groups

Variables	Groups				P	
	Control (N = 16)	POAG w/o DH (N = 16)	POAG w DH (N = 14)	POAG w/o DH vs. Control	POAG w DH vs. Control	POAG w/o DH vs. POAG w DH
Humphrey visual field						
MD (dB)	NA	-3.3 ± 2.7	-3.0 ± 3.2	NA	NA	0.78
PSD (dB)	NA	3.9 ± 2.7	4.6 ± 3.1	NA	NA	0.53
ONH parameters						
Average RNFL (µm)	96.5 ± 8.9	68.8 ± 16.1	73.7 ± 14.1	<0.001*	<0.001*	> 0.99
Horizontal LCD (µm)	393.5 ± 86.1	460.0 ± 128.4	417.7 ± 85.0	0.29	> 0.99	0.88
Glaucoma medications (% of eyes)						
Prostaglandin analogs	0.0	62.5	64.3	NA	NA	> 0.99
β blockers	0.0	68.8	57.1	NA	NA	> 0.99
Carbonic anhydrase inhibitors	0.0	50.0	50.0	NA	NA	> 0.99
α agonists	0.0	31.3	42.9	NA	NA	> 0.99
OCT angiography findings						
ONH VD (%)						
Superficial vasculature	41.8 ± 3.2	41.2 ± 5.0	42.3 ± 2.7	> 0.99	> 0.99	> 0.99
Deep vasculature	44.1 ± 5.1	39.3 ± 4.5	39.1 ± 4.3	0.02*	0.02*	> 0.99
ONH IOS (%)						
Superficial vasculature	38.5 ± 3.9	40.4 ± 6.5	41.0 ± 3.9	0.96	0.26	> 0.99
Deep vasculature	44.7 ± 6.9	38.4 ± 5.6	39.2 ± 6.2	0.02*	0.09	> 0.99
Peripapillary VD (%)	40.7 ± 1.7	38.4 ± 3.6	37.6 ± 2.2	0.09	<0.001*	> 0.99
Peripapillary IOS (%)	47.8 ± 2.5	43.7 ± 5.0	44.0 ± 2.6	0.02*	0.001*	> 0.99

Data are expressed as mean ±SD.

* Statistically significant difference, $P < 0.05$.

Superficial vasculature is defined as the vessels from internal limiting membrane to BM. Deep vasculature defined as vessels from BM to 390 µm below. BM indicates Bruch's membrane; DH, disc hemorrhage; IOS, integrated OCT angiography by ratio analysis signal; LCD, lamina cribrosa depth; MD, mean deviation; NA, not available; OCT, optical coherence tomography; ONH, optic nerve head; POAG, primary open-angle glaucoma; PSD, pattern standard deviation; RNFL, retinal nerve fiber layer; VD, vessel density; w, with; w/o, without.