Regional Vessel Density Reduction in the Macula and Optic Nerve Head of Patients With Pre-Perimetric Primary Open Angle Glaucoma

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Précis: Capillary and neuronal tissue loss occur both globally and with regional specificity in pre-perimetric glaucoma patients at the level of the optic nerve and macula, with perifovea regions affected earlier than parafovea areas.

Purpose: To investigate optic nerve head (ONH) and macular vessel densities (VD) and structural parameters assessed by optical coherence tomography angiography in pre-perimetric open angle glaucoma (ppOAG) patients and healthy controls.

Materials and Methods: In all, 113 healthy and 79 ppOAG patients underwent global and regional (hemispheric/quadrants) assessments of retinal, ONH, and macular vascularity and structure, including ONH parameters, retinal nerve fiber layer (RNFL) and ganglion cell complex (GCC) thickness. Comparisons between outcomes in ppOAG and controls were adjusted for age, sex, race, BMI, diabetes, and hypertension, with $P<0.05$ considered statistically significant.

Results: In ppOAG compared with healthy controls: RNFL thicknesses were statistically significantly lower for all hemispheres, quadrants, and sectors ($P<0.001$–0.041); whole image peripapillary all and small blood vessels VD were statistically significantly lower for all the quadrants ($P<0.001$–0.002), except for the peripapillary small vessels in the temporal quadrant (ppOAG: 49.66 (8.40), healthy: 53.45 (4.04); $P=0.843$); GCC and inner and full macular thicknesses in the parafoveal and perifoveal regions were significantly lower in all the quadrants ($P=0.000$–$P=0.033$); several macular VD were significantly lower ($P=0.006$–0.034), with the exceptions of macular center, parafoveal superior and inferior quadrant, and perifoveal superior quadrant ($P>0.05$).

Conclusions: In ppOAG patients, VD biomarkers in both the macula and ONH, alongside RNFL, GCC, and macular thickness, were significantly reduced before detectable visual field loss with regional specificity. The most significant VD reduction detected was in the peripheric (perifovea) regions. Macular and ONH decrease in VD may serve as early biomarkers of glaucomatous disease.

Key Words: pre-perimetric glaucoma, optical coherence tomography angiography, optic nerve head, macula

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A wealth of evidence has previously highlighted various diverse aspects of vascular and metabolic abnormalities in the ocular tissues of patients with primary open angle glaucoma (OAG).[1] It is currently unknown, however, to what extent retinal, macular, and/or optic nerve head (ONH) ischemia are a primary cause of retinal ganglion cell death, neuronal tissue thinning, and visual field (VF) loss. Previous studies demonstrate strong associations between multiple vascular biomarkers and OAG status, yet data are scarce in pre-perimetric OAG (ppOAG) patients who do not already have significant neuronal tissue loss. In this context, it is important for studies to investigate hemodynamics as early as possible during the disease process, emphasizing assessment before significant visual progression occurs. Ideally, these evaluations would be in conjunction with the earliest characteristic structural glaucomatous changes occurring in the retina, macula, and ONH.

Recent advancements in noninvasive ocular imaging applications have allowed for an ever more detailed evaluation of specific structural and hemodynamic elements within the different anatomic regions of the eye affected by glaucoma. Specifically, optical coherence tomography angiography (OCTA) provides a near-simultaneous assessment of various structural characteristics of the retina and ONH while also providing quantification of small blood vessel densities (VD) in the different anatomic regions. Since its inception, OCTA applications of VD have been widely used to investigate the role of capillary loss in retinal and

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ONH tissues, especially in the pathophysiology of glaucoma.[1] A consensus of evidence has found lower retinal VD, especially in peripapillary regions of the retina surrounding the ONH, in patients with OAG compared with controls.[2–9] It is not certain, however, if capillary loss occurs before significant tissue degradation and associated VF defects in patients with OAG, and/or to what extent these biomarkers may serve as reliable predictors of disease progression. In addition, literature is scarce regarding early VD changes in ppOAG patients and longitudinal studies of VD loss, creating a void in understanding early capillary dropout as a primary risk factor for glaucoma progression.

While a broad body of evidence has shown a reduction of OCTA-assessed VD in the peripapillary regions in glaucoma subjects, data are more conflicting regarding VD loss at the level of the macular regions.[1–9] Along with less available data, varying patient populations and differing disease status of participants across studies truncate the current understanding of macular VD loss in OAG. In addition, the VD reported[2–9] are frequently provided without vessel size differentiation and are often reported only for the global regions of assessed tissues without regional data shown for each hemifield, quadrant, and sector. Glaucoma is a disease that strikes specific regions early, with local tissue damage occurring before global averages register defects. It is therefore important to analyze the structure and VD in each hemifield, quadrant, and sector with the specificity of vessel size and anatomic location.

Strong evidence, although mostly statistical associations, demonstrate OCTA VD biomarkers and OAG are linked, yet scarce data are available that specifically demonstrate early VD loss and capillary dropout as a primary driver of OAG disease. Further, current data on macular VD in OAG is conflicting, and the regional specificity of capillary and neuronal tissue erosion in ppOAG patients is uncertain. This study, therefore, was designed to investigate differences in regional VD of various sizes and structural elements within retinal, macular, and ONH tissues assessed by OCTA in ppOAG patients versus healthy controls.

MATERIAL AND METHODS

In a prospective, cross-sectional, observational single-visit study, 79 ppOAG and 113 healthy controls free from eye disease were enrolled between March 2021 and June 2022 at the Icahn School of Medicine at Mount Sinai, New York, NY, USA. All study subjects signed a written informed consent before initiation of this study. The study was conducted in accordance with the Declaration of Helsinki, and the study protocol was approved by the Institutional Review Board of Icahn School of Medicine at Mount Sinai, New York, NY, USA (protocol code: Study-20-00198; date of approval: April 19, 2020). Only subjects older than 21 years of age with the ability to understand and sign the written informed consent were enrolled.

For each participant, 1 randomly selected (coin flip) qualified eye was included in the study. A qualified glaucoma specialist defined ppOAG eye status based on the presence of an open angle, characteristic structural glaucomatous damage. Patients were without significant detectable functional VF defects through glaucoma Hemifield tests as defined by within normal limit or borderline with normal reliability parameters (< 33% fixation losses, <20% false-positive, <20% false-negative) with global indices [mean deviation (MD) and pattern SD (PSD)] within the 95% confidence limits, regardless of intraocular pressure (IOP) level, with the fellow eye being either also ppOAG (study eye chosen by coin flip) or healthy. The healthy group eyes consisted of subjects with both eyes being free of any eye diseases including ppOAG.

Inclusion criteria for all subjects included age 21 years or older and no other eye diseases including cataract. Participants were excluded for the following reasons: refractive error > +9 Diopters and <–9 Diopters in spherical equivalent; evidence of exfoliation or pigment dispersion; eye disease other than glaucoma or other eye health concerns; use of eye medications (other than IOP lowering medications for glaucoma or eye lubricants for dry eye); neurological disease (Alzheimer disease, Parkinson disease, multiple sclerosis); psychosis or neurological diseases that could prevent reliable eye exams; severe, unstable or uncontrolled cardiovascular, renal, or pulmonary disease.

Patients were assessed during a single 2-hour study visit. Subject demographics, including age, self-reported race and ethnicity, biological sex, height, weight, diabetic status, hypertensive status, and ocular and systemic medication use,

TABLE 1. Mean and SD of Demographic, Ocular and Systemic Parameters in Healthy Control and Pre-perimetric Open Angle Glaucoma (ppOAG) Patients

	Subjects	Mean (SD)	*P*, Control vs. ppOAG
Age at visit (y)	Control	40.9 (16.6)	<0.001*
	ppOAG	67.3 (13.8)	—
BMI	Control	26.9 (6.6)	0.420
	ppOAG	26.9 (5.6)	—
IOP (mm Hg)	Control	14.3 (2.7)	0.875
	ppOAG	16.1 (4.7)	—
SBP (mm Hg)	Control	121.3 (15.6)	<0.001*
	ppOAG	131.2 (17.3)	—
DBP (mm Hg)	Control	76.2 (10.2)	0.319
	ppOAG	78.4 (11.1)	—
MAP (mm Hg)	Control	91.2 (11.0)	0.009*
	ppOAG	96.0 (11.5)	—
OPP (mm Hg)	Control	46.5 (7.5)	0.685
	ppOAG	48.2 (8.9)	—
SOPP (mm Hg)	Control	107.0 (15.5)	0.930
	ppOAG	115.7 (17.8)	—
DOPP (mm Hg)	Control	61.9 (10.4)	0.415
	ppOAG	62.5 (12.1)	—
MOPP (mm Hg)	Control	77.0 (11.0)	0.580
	ppOAG	80.3 (12.4)	—
Heart rate (bpm)	Control	71.3 (12.4)	0.235
	ppOAG	69.7 (12.8)	—
Visual field MD (decibel)	Control	−1.35 (1.72)	0.114
	ppOAG	−2.64 (2.97)	—
Visual field PSD (decibel)	Control	2.07 (1.61)	0.056
	ppOAG	3.57 (2.25)	—
Visual field VFI (%)	Control	98.16 (3.08)	0.096
	ppOAG	94.33 (6.64)	—

Comparisons between outcomes were adjusted for age, diabetes status, and BP.

*$P < 0.05$.

BMI indicates body mass index; bpm, beats per minute; DBP, diastolic blood pressure; DOPP, diastolic ocular perfusion pressure HR, heart rate; IOP, intraocular pressure; MAP, mean arterial pressure; MD, mean deviation; OPP, ocular perfusion pressure; PSD, pattern SD; SBP, systolic blood pressure; SOPP, systolic ocular perfusion pressure; VF, visual field; VFI, visual field index.

were recorded. One qualified study eye was randomly selected for each study subject (coin flip), and all the eye measurements were performed only in the study eye selected for each participant. Study participants underwent assessment for IOP through Goldmann applanation, systolic blood pressure (SBP) and diastolic blood pressure (DBP) (through automated ambulatory blood pressure monitor at rest), heart rate; mean arterial pressure (MAP) and ocular perfusion pressure (OPP) were calculated (MAP = [SBP + (2 × DBP)]/3; OPP = 2/3 × MAP − IOP). The visual function was assessed through Humphrey field analyzer II using the 24-2 Swedish interactive threshold algorithm standard (white III stimulus) V.4.1 (Carl Zeiss Mediatec, Dublin, CA, USA) and visual field index (VFI), MD, and PSD were recorded.

OCTA imaging was assessed in all study eyes using the RTVue XR Avanti System (RTVue XR, Version 2018.1.1.63, Optovue Inc, Fremont, CA, USA) that provides a noninvasive 3-dimensional visualization of the retinal microvasculature and OCT-derived structural parameters at the level of the ONH, retina and macula regions. The details on the principles of OCTA are available elsewhere.[10] In this study, we used the AngioAnalytics licensed upgrade, which automatically provides separate VD analysis computed as the percentage of area occupied by OCTA-detected vasculature at the level of the macula and ONH.[11] The VD was measured for the small vessels (ie, with large vessel masking) and for all vessels; the application of large vessel mask has a threshold of ≥3 pixels (~≥33 μm). The ONH VD was assessed using the 4.5 mm HD Angio Disk scan in the radial peripapillary capillary (RPC) slab from the internal limiting membrane to the nerve fiber layer in the following different regions: peripapillary region (defined by 2 rings of 2 mm and 4 mm centered on disk center); inside the optic disk; and in the entire region. The ONH VD information included were

TABLE 2. Mean and SD of the Optical Coherence Tomography Parameters and Comparisons (*P* values) in Healthy Control and Preperimetric Open Angle Glaucoma (ppOAG) Patients for the Optic nerve Head (ONH) and Retinal nerve Fiber Layer (RNFL)

	Subjects	Mean (SD)	*P*, Control versus ppOAG
ONH C/D area ratio	Control	0.30 (0.14)	<0.001*
	ppOAG	0.55 (0.17)	—
ONH C/D vertical ratio	Control	0.47 (0.16)	0.043*
	ppOAG	0.72 (0.15)	—
ONH C/D horizontal ratio	Control	0.57 (0.18)	0.108
	ppOAG	0.79 (0.15)	—
ONH rim area (mm^2)	Control	1.41 (0.26)	<0.001*
	ppOAG	0.87 (0.28)	—
ONH disk area (mm^2)	Control	1.99 (0.38)	0.069
	ppOAG	2.00 (0.43)	—
ONH Cup volume (mm^3)	Control	0.11 (0.12)	0.007*
	ppOAG	0.36 (0.30)	—
RNFL thickness average (μm)	Control	101.65 (8.56)	<0.001*
	ppOAG	80.71 (11.73)	—
RNFL thickness superior hemisphere (μm)	Control	103.97 (10.09)	<0.001*
	ppOAG	82.83 (12.54)	—
RNFL thickness inferior hemisphere (μm)	Control	99.37 (8.06)	<0.001*
	ppOAG	78.50 (13.45)	—
RNFL thickness superior quadrant (μm)	Control	126.78 (13.67)	<0.001*
	ppOAG	97.96 (16.85)	—
RNFL thickness nasal quadrant (μm)	Control	77.63 (10.36)	0.001*
	ppOAG	65.53 (14.14)	—
RNFL thickness inferior quadrant (μm)	Control	128.04 (12.02)	<0.001*
	ppOAG	96.39 (24.95)	—
RNFL thickness temporal quadrant (μm)	Control	74.06 (10.28)	0.002*
	ppOAG	63.61 (14.14)	—
RNFL thickness supero nasal sector (μm)	Control	116.04 (17.48)	<0.001*
	ppOAG	88.30 (21.36)	—
RNFL thickness nasal upper sector (μm)	Control	82.21 (12.24)	0.002*
	ppOAG	68.56 (14.13)	—
RNFL thickness nasal lower sector (μm)	Control	73.24 (10.50)	0.002*
	ppOAG	62.53 (15.31)	
RNFL thickness infero nasal sector (μm)	Control	119.66 (21.05)	<0.001*
	ppOAG	89.39 (23.62)	—
RNFL thickness infero temporal sector (μm)	Control	138.03 (15.01)	<0.001*
	ppOAG	103.47 (23.57)	—
RNFL thickness temporal lower sector (μm)	Control	68.18 (10.21)	0.001*
	ppOAG	60.03 (17.56)	—
RNFL thickness temporal upper sector (μm)	Control	80.34 (18.14)	0.041*
	ppOAG	67.50 (14.36)	—
RNFL thickness supero temporal sector (μm)	Control	138.20 (15.73)	<0.001*
	ppOAG	109.83 (22.31)	—

C/D indicates cup-to-disk.
*$P < 0.05$.

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RPC VD (global and peripapillary in the superior and inferior hemisphere) for both the small vessels and all vessels; regional vessel density (small vessels only) for the peripapillary region in the superior, temporal, nasal, and inferior quadrants. The retinal thicknesses and vessel density were assessed using the 6.0 mm HD AngioRetina scan in the Early Treatment Diabetic Retinopathy Study grid comprised of 3 concentric rings: 1 mm (macular center), 1–3 mm (parafovea), and outer ring of 3–6 mm diameters (perifovea).[11] In addition to the measurements of the OCTA parameters, all study participants were assessed for the thickness of the peripapillary retinal nerve fiber layer (RNFL) (average; hemisphere: superior and inferior hemisphere; quadrants: superior, inferior, temporal, and nasal; and sectors: supero nasal; nasal upper; and nasal lower; infero nasal and infero temporal; temporo lower; temporal upper; superior temporal; for ONH structural parameters (cup (C)/disk (D) area ratio, C/D vertical ratio, C/D horizontal ratio, rim area, disk area, cup volume); for inner and full retinal thickness in the macular center, parafoveal and perifoveal regions (average; quadrants: superior, inferior, temporal, and nasal); and for parameters related to the macular ganglion cell complex (GCC) represented by the 3 innermost layers ganglion cell layer, inner plexiform layer, and retinal nerve fiber layer (GCC thickness total, inferior, and superior; global loss volume and focal loss volume). Only images with optimal image quality (signal strength index for the OCT scans >50; scan quality index for the OCTA scans >6) were included in the analysis.

Comparisons for differences in biomarkers between ppOAG and healthy subject outcomes were performed using nonparametric tests, with adjustment for age, sex, race, body mass index level, diabetes status, and systemic arterial hypertension/high blood pressure status. A 2-sided 5% significance level was used. Statistical analyses were performed using SAS (SAS Institute Inc., Cary, NC).

RESULTS

Demographic, ocular, and systemic parameters for OAG patients and healthy controls are shown in Table 1. Age, diabetes status, hypertension status, SBP, and MAP were significantly different between glaucoma and healthy control subjects (Table 1). Because of those differences, comparisons between outcomes were statistically adjusted for age, diabetes status, and hypertension/high blood pressure status. No statistically significant differences were found between ppOAG and healthy subjects for VF parameters MD, PSD, and VFI (Table 1).

Table 2 shows the OCT-derived ONH and RNFL in the 2 groups. Average RNFL thickness was significantly lower in patients with ppOAG (mean: 80.71 (SD, SD:11.73) vs. controls [101.65 (8.56); $P<0.001$] while C/D vertical ratio was higher in ppOAG patients [0.72 (0.15) vs. 0.47 (0.16); $P=0.043$]. The RNFL thickness was also significantly lower in ppOAG compared with healthy controls for all hemispheres, quadrants, and sectors ($P<0.001$-0.041), as detailed in Table 2 and shown in Figure 1.

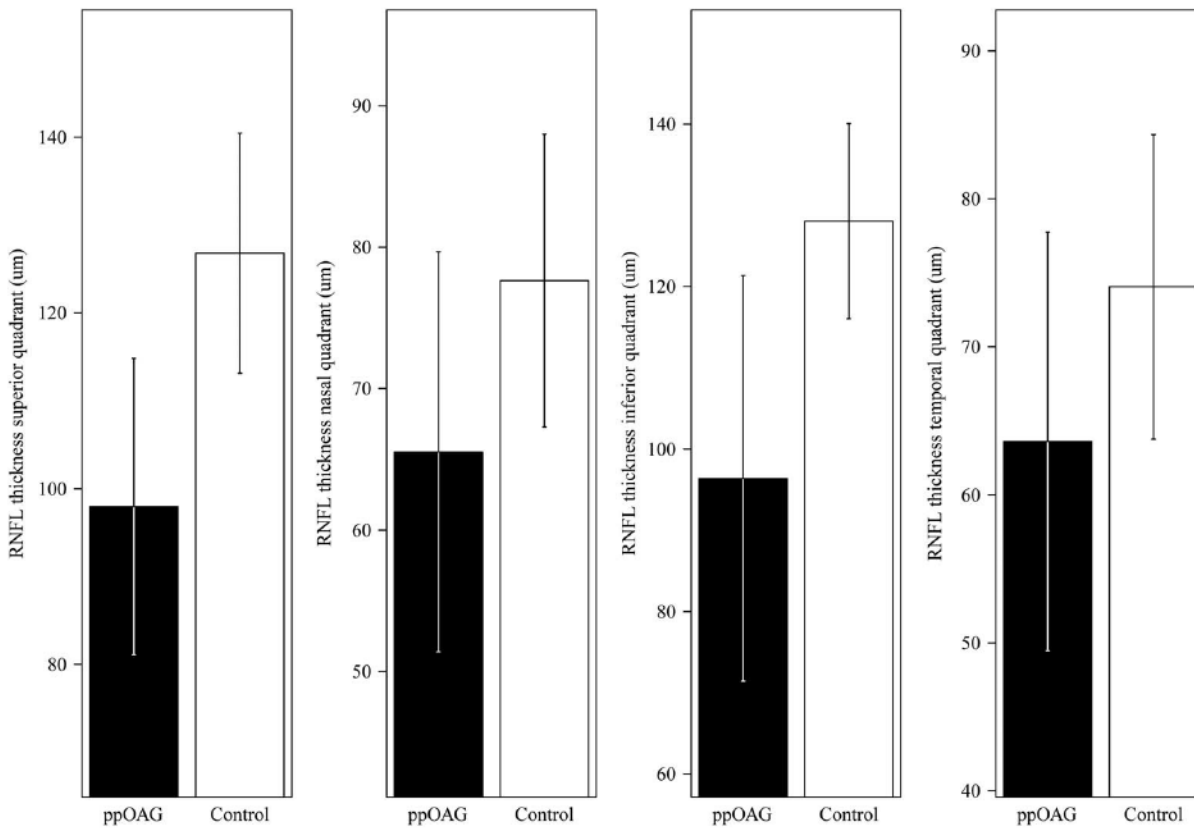

FIGURE 1. Mean ± SD retinal nerve fiber layer (RNFL) thicknesses by quadrant in healthy control and pre-perimetric open angle glaucoma (ppOAG) patients. All $P<=0.002$. ppOAG indicates pre-perimetric open angle glaucoma; RNFL, retinal nerve fiber layer.

Within the ONH, whole image small blood vessels VD and global peripapillary VD were significantly lower in ppOAG patients versus controls [43.99 (4.49) vs. 49.3 (2.5); $P<0.001$, Table 3] and [45.69 (6.02) vs. 51.89 (3.10); $P<0.001$]. The peripapillary VD of the SV was also statistically significantly lower in ppOAG versus controls for all quadrants ($P<0.001$–0.002), except for the temporal quadrant where the difference was not statistically significant [ppOAG: 49.66 (8.40), healthy: 53.45 (4.04); $P=0.843$], as shown in Figure 2.

Biomarkers of macular thicknesses in the parafoveal and perifoveal regions were significantly lower in ppOAG patients compared with healthy controls in all quadrants ($P<0.001$–$P=0.033$), Table 4, Figures 3 and 4. GCC thicknesses were also significantly lower in ppOAG patients compared with controls [average: 95.4 (7.03) vs. 84.95 (15.62); $P<0.001$]; [superior: 94.87 (7.95) vs. 83.68 (11.59); $P<0.001$]; [inferior: 95.84 (7.44) vs. 86.31 (27.51); $P<0.001$], Table 4 and Figure 5.

Multiple VD biomarkers were statistically significantly lower in ppOAG compared with healthy in the parafoveal and perifoveal regions, with exceptions of VD in the macular center, in the superior quadrant perifoveal regions, and in the inferior and superior quadrant in the parafoveal regions, although all were nonsignificantly lower in ppOAG subjects compared with controls (Table 5 and Figure 6).

DISCUSSION

A large body of evidence has linked multi-aspects of ocular hemodynamic and metabolic insult with OAG; however, the primary nature of vascular dysfunction in glaucoma remains to be determined.[1] By the time detectable VF loss occurs in OAG, significant tissue erosion would be expected, as might a reduction in the vascularity of those tissues. By investigating VD reduction and loss of perfusion before the disease reaches perimetric detection, the primary versus secondary nature of loss of vascularity and ocular blood flow may be further revealed. Due to the nature of glaucoma, a focus should also be made on hemodynamic data rigor, with transparency and specificity of data shown inclusive of determinates of size, sector, region, and hemifield.

Our results in the current investigation are novel as they reflect very early glaucomatous changes before significant VF loss and include vessel size differentiation, which are both scarce in current literature. In addition, we report VD parameters for the whole optic nerve head and inside disk regions, alongside the more frequently reported optic nerve head and peripapillary regions, while also analyzing both perifoveal and parafovea macular regions with quadrants differentiation. This approach allows us to simultaneously assess structural and hemodynamic damage at a sectorial, quadrant, and hemispheric level. This is important as OAG is a disease in which regional tissue damage may occur before changes in the global values.

TABLE 3. Mean and SD of the Optical Coherence Tomography Angiography (OCTA) Vessel Density (VD) and comparisons (*P* values) in Control and Pre-Perimetric Open Angle Glaucoma (ppOAG) Patients

Vessel density (%)	Subjects	Mean (SD)	*P*, Control vs. ppOAG
Whole image of all vessels	Control	55.55 (2.73)	<0.001*
	ppOAG	49.62 (4.55)	—
Inside disk of all vessels	Control	64.63 (50.35)	0.240
	ppOAG	55.71 (6.74)	—
Peripapillary global of all vessels	Control	57.88 (3.20)	<0.001*
	ppOAG	50.47 (8.07)	—
Peripapillary superior hemisphere of all vessels	Control	58.36 (3.44)	<0.001*
	ppOAG	51.81 (6.27)	—
Peripapillary inferior hemisphere of all vessels	Control	57.42 (3.32)	0.001*
	ppOAG	50.35 (6.55)	—
Whole Image of small vessels	Control	49.30 (2.50)	<0.001*
	ppOAG	43.99 (4.49)	—
Inside disk of small vessels	Control	50.40 (5.63)	0.637
	ppOAG	47.70 (7.71)	—
Peripapillary global of small vessels	Control	51.89 (3.10)	<0.001*
	ppOAG	45.69 (6.02)	—
Peripapillary superior hemisphere of small vessels	Control	52.08 (3.53)	<0.001*
	ppOAG	45.59 (8.04)	—
Peripapillary inferior hemisphere of small vessels	Control	51.66 (3.15)	0.002*
	ppOAG	44.90 (6.91)	—
Peripapillary superior quadrant of small vessels	Control	52.59 (4.09)	0.002*
	ppOAG	45.79 (7.30)	—
Peripapillary nasal quadrant of small vessels	Control	48.93 (4.48)	<0.001*
	ppOAG	42.28 (6.63)	—
Peripapillary inferior quadrant of small vessels	Control	53.98 (3.72)	<0.001*
	ppOAG	46.24 (6.95)	—
Peripapillary temporal quadrant of small vessels	Control	53.45 (4.04)	0.843
	ppOAG	49.66 (8.40)	—

The optic nerve head (ONH) VD represents the percentage of area occupied by OCTA-detected vasculature in the radial peripapillary capillary slab in 3 regions: peripapillary region (defined by 2 rings of 2 mm and 4 mm centered on disk center); inside the optic disk ("Inside Disk"); and entire region ("Whole Image"). The VD are measured for all vessels and for the small vessels (ie, with large vessel masking).

*$P<0.05$.

Using OCTA applications, we simultaneously evaluated the regional structural and VD characteristics of persons with early structural characteristics of OAG but without detectable VF loss compared with healthy subjects without glaucoma at the level of the peripapillary retina, ONH, and macula. In our study, patients with ppOAG and healthy controls did not significantly differ in terms of visual function, including measures of VF global indices MD, PSD and/or VFI ($P > 0.05$, Table 1). Subjects with ppOAG demonstrated characteristics of structural damage indicative of glaucoma at the level of the ONH (statistically significant higher c/d area ratio, c/d vertical ratio, and cup volume, and lower rim area, compared with healthy subjects, Table 2) and RNFL (statistically significant lower global and regional RNFL thicknesses globally and in all quadrants and sectors compared with healthy subjects, Table 2 and Fig. 1). Our results agree with previous pilot work showing characteristic of structural changes at the level of the ONH and RNFL in patients with ppOAG and in the absence of VF defects as detectable by standard automated perimetry.[2,12,13] Well-powered longitudinal studies are needed to investigate if the reductions of VD in the macula, peripapillary, and ONH regions observed in our and other ppOAG patients are predictive of future functional glaucoma progression and how the specific aspects of VF damage relate to the specific regions of VD loss, capillary dropout, and local tissue thinning.

In these ppOAG patients, ONH and macular global and regional VD and structural (tissue thickness) assessed by OCTA were all significantly lower compared with healthy controls. Both controls and ppOAG had statistically similar VF detection, suggesting that macular and ONH capillary loss occurs before detectible visual dysfunction. In addition, our regional specific data show that standard outpatient assessment exams that include VFs and global averages of structure may mask regional VD loss and localized structural erosion. The capability of assessing sector-specific loss of VD during a noninvasive outpatient exam provides a translational framework for using OCTA VD as early biomarkers of glaucomatous damage alongside the structural changes occurring within the same anatomic regions.

Within anatomic regions of interest, OCTA-derived parameters at the level of the ONH for the whole image and peripapillary region were all significantly lower in ppOAG patients versus controls for both all vessels and small vessels. This was true both globally and on a regional level (hemisphere and quadrants) except for the temporal quadrant, where the difference was not statistically significant (Table 3 and Fig. 2). These results confirm previous studies that showed reduced ONH VD in ppOAG patients, and together suggest that impaired hemodynamics at the level of the ONH is an early sign of OAG disease in patients without VF loss.[2,12,14] Current data in ppOAG are limited but, in general, agree with our findings. Akil et al previously showed a statistically significant difference between control and ppOAG eyes for peripapillary, ONH, and papillary VD values; however, their analyses were limited to global

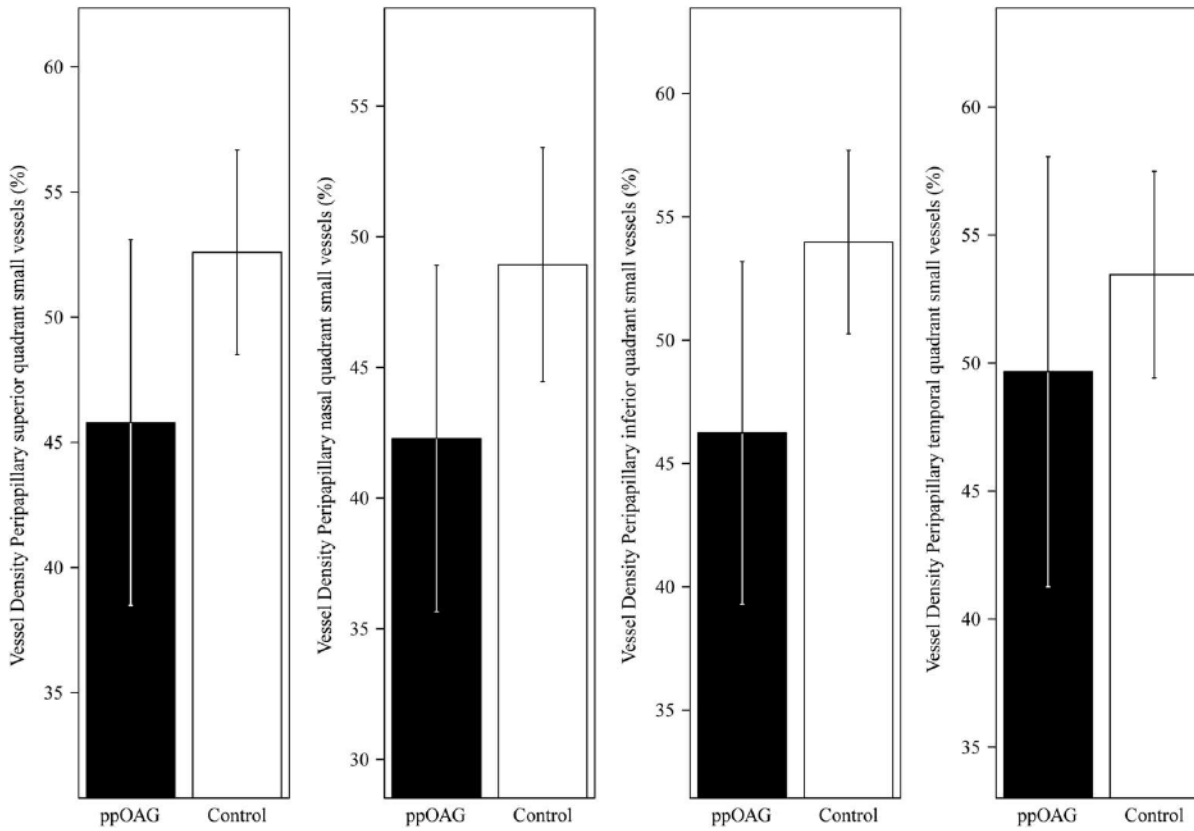

FIGURE 2. Mean ± SD peripapillary vessel densities for small vessels by quadrant in healthy control and pre-perimetric open angle glaucoma (ppOAG) patients. $P < = 0.002$ for superior, nasal, and inferior quadrants, not significant ($P = 0.843$) for temporal quadrant. ppOAG indicates pre-perimetric open angle glaucoma.

averages for the peripapillary and papillary area and only to the inferior and superior hemispheres in the papillary area.[2] Similarly, the investigation of Cennamo et al who found significantly lower flow index ($P=0.021$) and vessel density ($P=0.001$) in the papillary region of ppOAG compared with healthy eyes was also limited to the global papillary region.[12] In our results, we found significantly lower VD in the superior and inferior hemispheres, and in the superior, inferior, and nasal quadrants in ppOAG eyes compared with healthy, while the VD in the temporal did not differ significantly among groups, thus suggesting that the impairment of blood flow may occur at a regional level. This is important as OAG may affect each individual differently, with vascular dysfunction for each person and VD reduction occurring within different regions of the retina, macula, and/or ONH. Interestingly, in our study, we did not find differences in VD in the region inside the disk ($P>0.05$, Table 3). These findings do agree with the data of Chen et al (inside disk RPC % control 51.2 ± 5.9; ppOAG: 50.4 ± 5.6; $P=0.432$[13]) but differ compared with Akil et al[2] (optic nerve head vessel density (%): control 86.6 ± 4.7; ppOAG: 78.04 ± 7.2; $P<0.001$). These differences may be explained by variance in imaging devices used: both in ours and Chen's study, the Optovue OCTA was used, while Akil et al

TABLE 4. Mean and SD of the Optical Coherence Tomography Parameters and Comparisons (*P* values) in Healthy Control and Preperimetric Open Angle Glaucoma (ppOAG) Patients for Macular Parameters

	Subjects	Mean (SD)	*P*, Control versus ppOAG
GCC thickness average (μm)	Control	95.40 (7.03)	<0.001*
	ppOAG	84.95 (15.62)	—
GCC thickness superior hemisphere (μm)	Control	94.87 (7.95)	<0.001*
	ppOAG	83.68 (11.59)	—
GCC thickness inferior hemisphere (μm)	Control	95.84 (7.44)	<0.001*
	ppOAG	86.31 (27.51)	—
FLV	Control	1.39 (2.42)	0.001*
	ppOAG	5.30 (5.11)	—
GLV	Control	3.83 (4.19)	<0.001*
	ppOAG	14.15 (8.58)	—
	ppOAG	258.79 (31.27)	—
Parafovea full thickness average (μm)	Control	315.73 (18.44)	0.033*
	ppOAG	301.22 (29.40)	—
Parafovea full thickness superior quadrant (μm)	Control	327.55 (17.40)	0.002*
	ppOAG	306.89 (24.43)	—
Parafovea full thickness nasal quadrant (zμm)	Control	327.65 (17.59)	0.004*
	ppOAG	309.36 (23.60)	—
Parafovea full thickness inferior quadrant (μm)	Control	324.37 (16.81)	0.005*
	ppOAG	301.49 (25.10)	—
Parafovea full thickness temporal quadrant (μm)	Control	314.81 (16.29)	<0.001*
	ppOAG	296.83 (20.88)	—
Perifovea full thickness average (μm)	Control	286.50 (15.99)	0.024*
	ppOAG	267.06 (20.38)	—
Perifovea full thickness superior quadrant (μm)	Control	285.25 (15.44)	0.003*
	ppOAG	265.24 (20.91)	—
Perifovea full thickness nasal quadrant (μm)	Control	301.00 (16.88)	0.005*
	ppOAG	277.20 (23.05)	—
Perifovea full thickness inferior quadrant (μm)	Control	274.63 (14.32)	<0.001*
	ppOAG	253.58 (18.29)	—
Perifovea full thickness temporal quadrant (μm)	Control	268.96 (14.47)	<0.001*
	ppOAG	253.35 (16.63)	—
Parafovea inner thickness superior quadrant (μm)	Control	111.50 (9.21)	<0.001*
	ppOAG	93.36 (18.51)	—
Parafovea inner thickness nasal quadrant (μm)	Control	109.92 (9.22)	<0.001*
	ppOAG	92.19 (17.25)	—
Parafovea inner thickness inferior quadrant (μm)	Control	112.16 (8.80)	<0.001*
	ppOAG	90.72 (21.39)	—
Parafovea inner thickness temporal quadrant (μm)	Control	101.07 (8.61)	<0.001*
	ppOAG	85.06 (15.78)	—
Perifovea inner thickness superior quadrant (μm)	Control	97.96 (8.83)	<0.001*
	ppOAG	84.11 (15.91)	—
Perifovea inner thickness nasal quadrant (μm)	Control	115.80 (10.02)	<0.001*
	ppOAG	98.43 (12.42)	—
Perifovea inner thickness inferior quadrant (μm)	Control	97.21 (8.08)	<0.001*
	ppOAG	78.83 (14.49)	—
Perifovea inner thickness temporal quadrant (μm)	Control	84.46 (6.63)	<0.001*
	ppOAG	73.22 (11.02)	—

*$P<0.05$.
FLV indicates focal loss volume; GCC, ganglion cell complex; GLV, global loss volume.

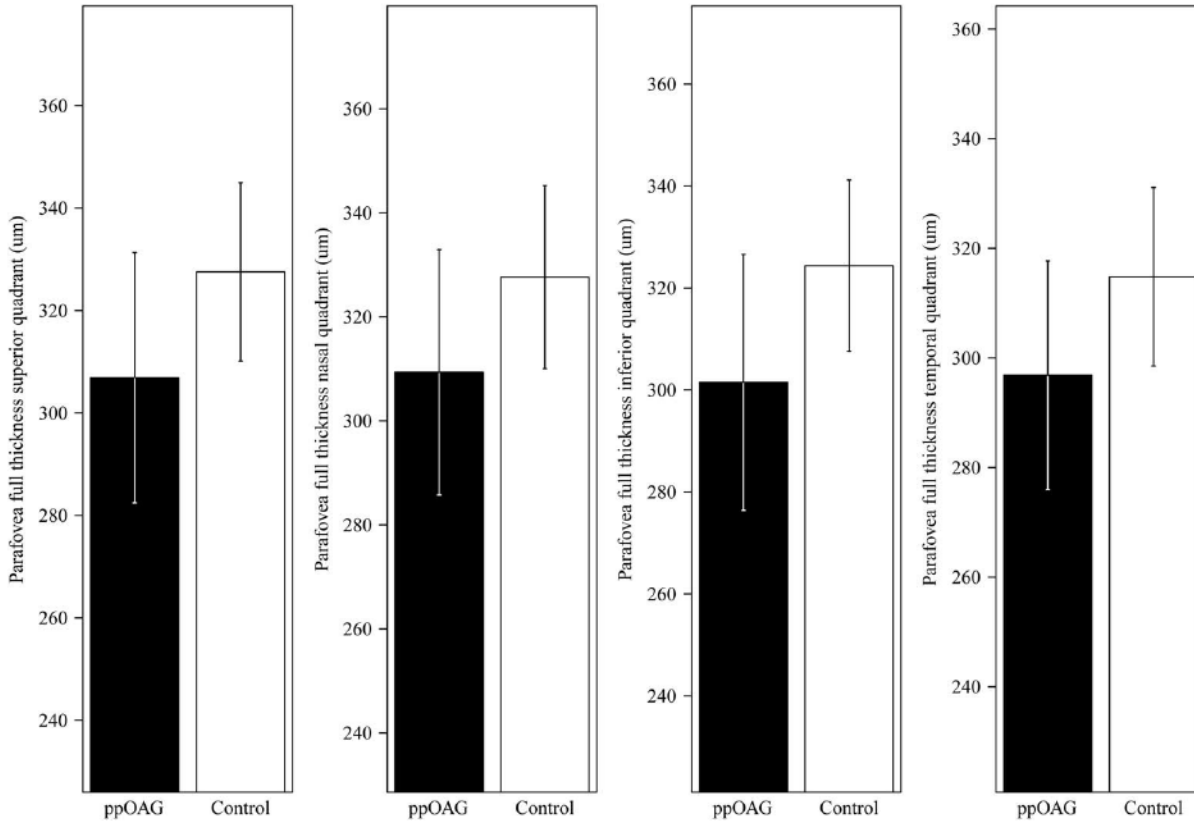

FIGURE 3. Mean ± SD parafovea full thicknesses by quadrant in healthy control and pre-perimetric open angle glaucoma (ppOAG) patients. All $P <= 0.005$. ppOAG indicates pre-perimetric open angle glaucoma.

utilized a Topcon OCTA device (DRI OCT Triton, Topcon). These results thus also indicate that the use of different OCTA devices with differing acquisition protocols (3×3 mm scan vs. 4.5×4.5 mm scan) and software versions may significantly influence study results. Caution is therefore suggested when comparing data derived from different OCTA devices or algorithms, and their measurements should not be used interchangeably.

In addition to ONH and peripapillary (ie, RNFL) parameters, macular structural parameters have been investigated as potential early indicators of glaucoma. In humans approximately 50% of the over 1 million retinal ganglion cells (RGC) are located within 4.5 mm of the fovea, and up to 50% of RGCs may be lost before VF loss is detectable.[15–18] In our study, macular structural parameters were significantly lower in ppOAG compared with healthy subjects without OAG at both a global and regional level, both considering the inner retina and the full retinal thickness (Table 4 and Figs. 3, 4 and 5). In detail, global and hemispheric GCC thicknesses (representing the 3 innermost retinal layers including ganglion cell layer, inner plexiform layer, and retinal nerve fiber layers) were statistically significantly lower compared with healthy subjects (Table 4 and Fig. 5). In addition, we also showed that ppOAG had higher GCC indices indicative of global and focal loss volumes compared with healthy subjects (Table 4). These data agree with previous results that showed thinning of the GCC in pre-perimetric glaucoma patients.[12,13,19] We also found that the full macular thickness in the parafoveal and perifoveal regions were significantly lower in the ppOAG patients compared with the healthy subjects in all quadrants (Table 4), thus both confirming and adding novel information to results from previous studies that were limited to global and hemispheric analyses.[13]

Our ppOAG patients also had significantly lower regional macular VD compared with controls. Macular center, the superior quadrant (parafoveal and perifoveal regions), and the inferior quadrant (parafoveal regions) were also all numerically lower in ppOAG but not statistically significant (Table 5 and Fig. 6). Previously, Hou et al investigated the differences in macular vessel densities in 57 healthy and 68 ppOAG in a multivariate analysis adjusted for race, sex, age, self-reported hypertension and diabetes status, and scan quality, and showed a statistically significant reduction of VD in the perifoveal regions for all the 4 quadrants, including the superior one [healthy: 51.6 (50.8, 52.4); ppOAG: 49.0 (48.1, 49.9), $P = 0.007$].[20] In our study, the parafoveal region was shown to be less involved in the microvascular damage possibly due to a higher vulnerability of the perifoveal region, with statistically significant capillary loss in ppOAG compared with healthy subjects only in the temporal ($P = 0.006$) and nasal ($P = 0.034$) quadrants and no statistically significant for the superior ($P = 0.095$) and inferior ($P = 0.261$) quadrants. Similarly, Lu et al investigated the macular VD in 41 healthy and 44 ppOAG and showed extensive VD reduction at the level of the perifoveal region with significantly lower VD in ppOAG compared with healthy for all 4 quadrants (all $P < 0.05$).[14] In this study, only the temporal quadrant of the parafoveal region showed a significant reduction of the VD in ppOAG compared with healthy ($P = 0.044$),

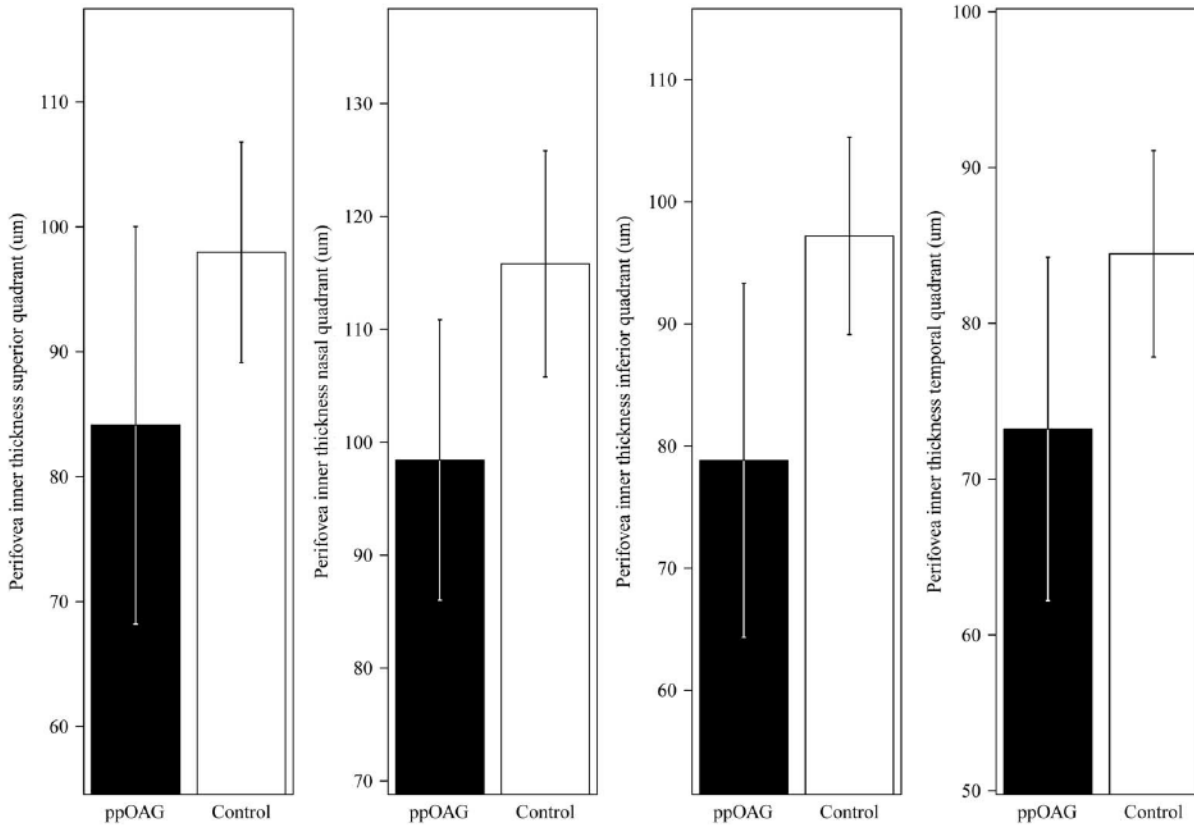

FIGURE 4. Mean ± SD perifovea inner thicknesses by quadrant in healthy control and pre-perimetric open angle glaucoma (ppOAG) patients. All $P < 0.001$. ppOAG indicates pre-perimetric open angle glaucoma.

while no statistically significant differences were found in the superior ($P = 0.453$), nasal ($P = 0.263$) and inferior ($P = 0.237$) quadrants between the 2 groups. However, when comparing eyes with early perimetric glaucoma to ppOAG, the perifoveal ($P = 0.001$–0.029) and the parafoveal VD ($P = 0.001$–0.009) were significantly lower in all quadrants in eyes with detectable VF damage compared with those without.[14] Taken together, these results suggest that loss of capillaries occurs first in the peripheral region (perifovea) of the macula when the disease is pre-perimetric, and then extends centrally (parafovea) as the disease progresses to a perimetric stage.

Our study showed a more consistent pattern of structural loss and corresponding VD reduction at the level of the peripapillary region compared with the macula. The pattern of damage was consistent between structural loss of RNFL (significant thinning of RNFL thickness in all hemispheres and sectors) and reduction of VD in all hemispheres and sectors except for only the temporal quadrant where the structural damage was evident without apparent hemodynamic impairment (Tables 2 and 3, Fig. 1 and 2). A study from Yu et al. using speckle variance OCT showed a positive correlation between RPC density and RNFL thickness in the human retina.[21] A previous longitudinal analysis of patterns of RNFL progression over 36 months found the infero temporal quadrant was the most frequent location where progression was detected.[22] While the regional thinning of the RNFL corresponded well with VD reduction in the same locations, we showed a mismatch between the structural macular damage which was present in all the quadrants (Table 4, Figs. 3 and 4) compared with the reduced VD, which occurred only in some of the analyzed regions (Table 5 and Fig. 6). These results suggest that the neurodegenerative process at the level of the macula and ONH and the relationship between structural and vascular damage may follow different patterns in these 2 different anatomic regions and should be assessed independently of each other with regional specificity.

Our study has several significant limitations to acknowledge, including ppOAG patients were significantly older than healthy subjects by an average of 17 years (Table 1). This is important as studies as increased age is associated with a reduction of RNFL thickness and VD at the level of the macula and ONH,[23,24] thus enhancing the differences between the 2 groups. To limit the impact our results were statistically adjusted for age, sex, race, body mass index level, diabetes status, and systemic arterial hypertension. The inclusion of these patient characteristics as covariates in the analysis provides a comparison between the ppOAG and the control group, with the other characteristics held constant. While a study could be designed to match groups on additional patient characteristics, such a design would be difficult in this prospective study that includes extensive measurements that are not part of standard clinical eye care. It is also important to note that differences in variables, including refractive error, high myopia, peripapillary atrophy, lens status, and OCT/OCTA quality score, may affect outcomes. However, our study participants did

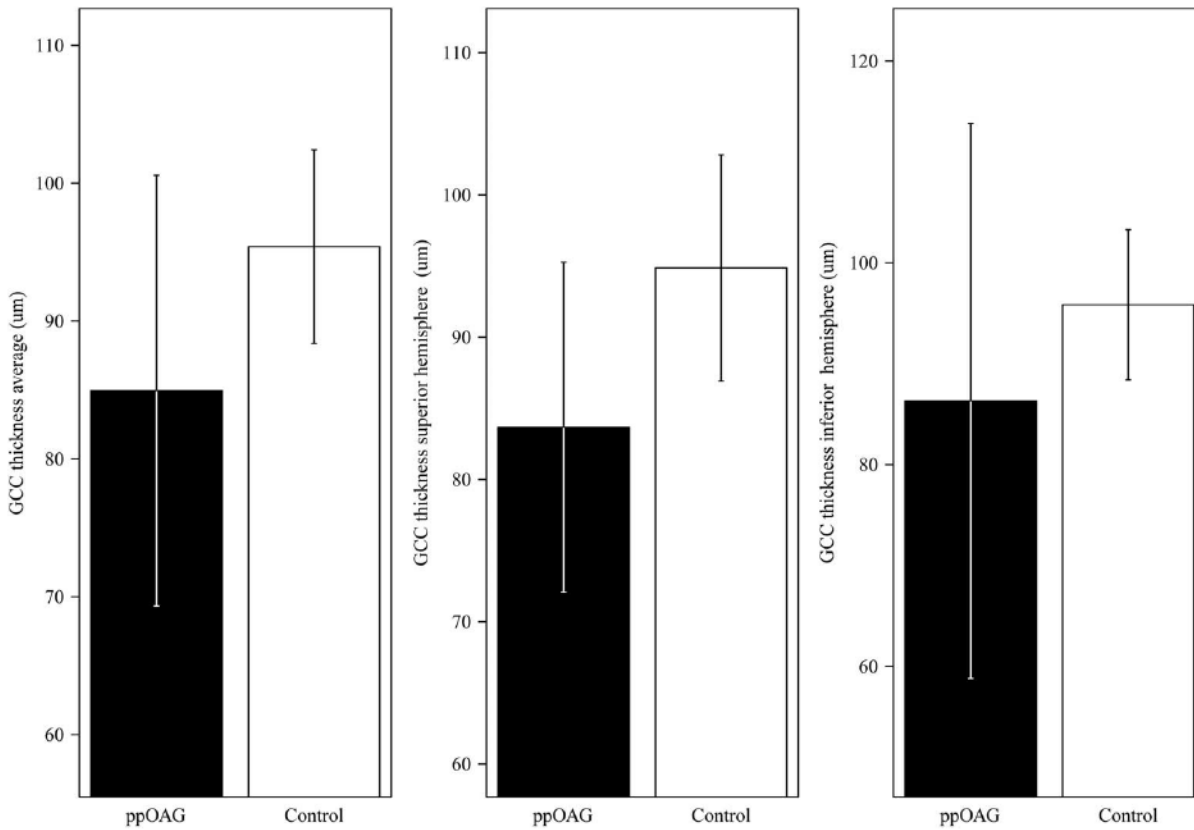

FIGURE 5. Mean ± SD ganglion cell complex (GCC) thicknesses (average, superior and inferior hemispheres) in healthy control and pre-perimetric open angle glaucoma (ppOAG) patients. All $P < 0.001$. GCC indicates ganglion cell complex; ppOAG, pre-perimetric open angle glaucoma.

not significantly differ in these variables as they only had mild refractive errors, were without peripapillary atrophy or cataracts, and had similar high-quality OCTA scans between groups.

It is important to note that our study was cross-sectional and exploratory, and we, therefore, did not evaluate the role of VD biomarkers in the progression of the disease. Our study also investigated standard OCTA VD biomarkers and did not

TABLE 5. Mean and SD of the Optical Coherence Tomography Angiography (OCTA) Retinal Vessel Density (VD) and Comparisons (*P* values) in Control and Pre-perimetric Open Angle Glaucoma (ppOAG) Patients in 3 Concentric Rings: 1 mm (macular center), 1–3 mm (parafovea), and 3–6 mm diameters (perifovea)

	Subjects	Mean (SD)	*P*, Control versus ppOAG
VD macular center (%)	Control	19.45 (7.96)	0.521
	ppOAG	17.96 (7.80)	—
VD parafovea superior quadrant (%)	Control	52.38 (5.60)	0.095
	ppOAG	44.51 (9.48)	—
VD parafovea nasal quadrant (%)	Control	50.56 (6.75)	0.034*
	ppOAG	43.19 (8.26)	—
VD parafovea inferior quadrant (%)	Control	52.08 (5.95)	0.261
	ppOAG	44.00 (9.37)	—
VD parafovea temporal quadrant (%)	Control	51.62 (5.13)	0.006*
	ppOAG	43.04 (7.62)	—
VD perifovea superior quadrant (%)	Control	50.02 (5.04)	0.105
	ppOAG	44.17 (9.16)	—
VD perifovea nasal quadrant (%)	Control	54.10 (3.84)	0.028*
	ppOAG	47.21 (7.61)	—
VD perifovea inferior quadrant (%)	Control	49.81 (4.89)	0.027*
	ppOAG	41.41 (8.18)	—
VD perifovea temporal quadrant (%)	Control	45.72 (4.98)	0.006*
	ppOAG	38.63 (6.21)	—

*$P < 0.05$.

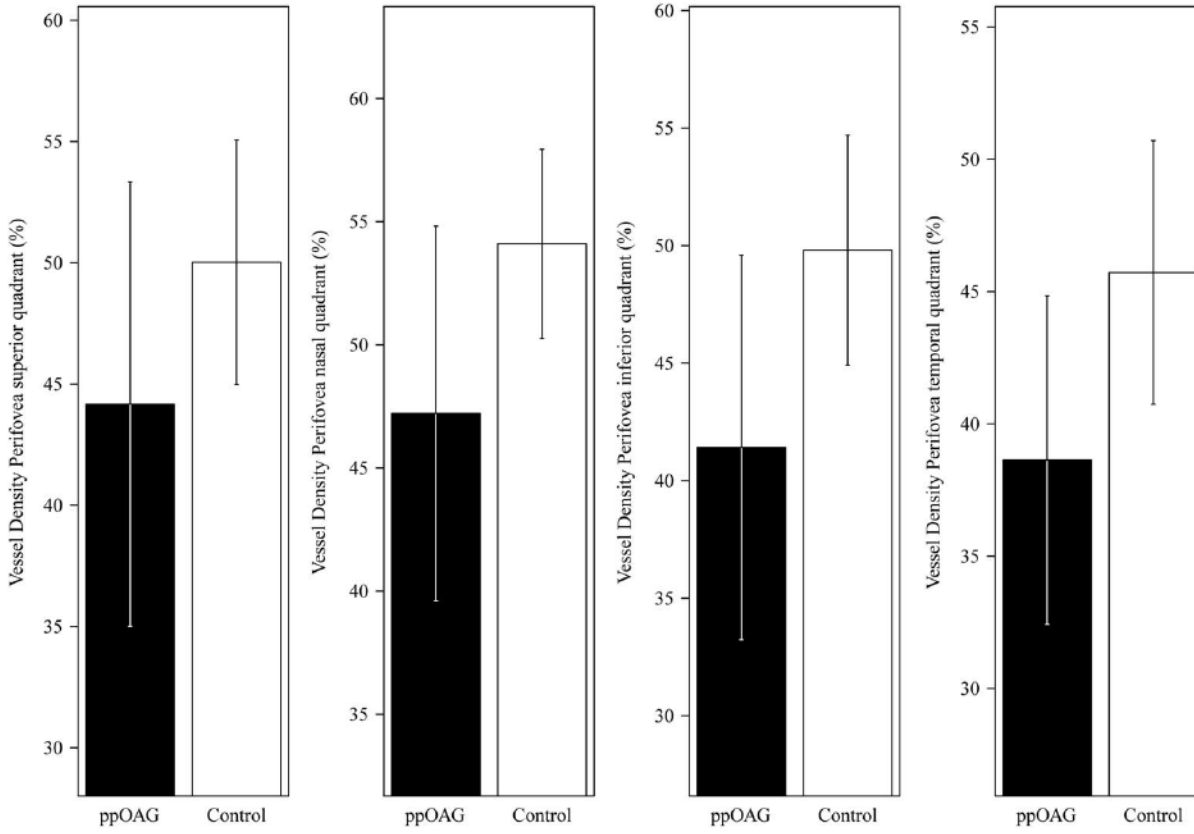

FIGURE 6. Mean ± Standard Deviation perifovea vessel densities by quadrant in healthy control and pre-perimetric open angle glaucoma (ppOAG) patients. $P < 0.03$ for nasal, inferior, and temporal quadrants, not significant ($P = 0.105$) for superior quadrant. ppOAG indicates pre-perimetric open angle glaucoma.

report on all the physiological aspects involved in capillary dropout. Previously, in 84 normotensive ppOAG patients for a period of 16 months, patients who progressed in their disease had reduced inferior ONH capillary blood flow assessed using laser speckle flowgraphy that was associated with specific superior sector VF changes using Garway-Heath mapping.[25] In addition, rapid functional progression has been associated with a weaker ONH tissue vasoreactivity,[26] which was not assessed in the current study. Only longitudinal studies properly designed to account for these differences and also other influencing factors in ppOAG over time have the ability to best inform on the role of reduced VD as a predictor of glaucoma functional and structural progression.

In conclusion, in this sample of ppOAG patients, global and regional VD were lower in both the macula and ONH alongside structural GCC, RNFL, and macular thinning before detectable VF loss. Specifically, within the macula, the capillary loss was more pronounced in the peripheric (perifovea) region compared with the parafovea, possibly due to a higher vulnerability of the peripheral microvasculature to glaucomatous damage.[14] These results highlight the presence of regional capillary loss and structural damage in glaucoma patients without functional defects and show that OCTA-derived biomarkers of regional macular and ONH capillary loss occur before detectible functional damage and may, therefore, play a predictive role for glaucoma. As global parameters outcomes may mask regional damage, our results suggest the importance of also including regional biomarkers in glaucoma risk assessment. Our results also show non-invasive OCTA VD biomarkers in the macula and ONH regions may have the potential to help screen subjects at high risk for glaucoma. Well-controlled longitudinal studies, including vasoreactive testing, are suggested to build upon these findings and test the predictive value of OCTA-derived VD biomarkers to determine the risk of progression from a pre-perimetric to a perimetric stage of the disease. The integration of artificial intelligence, machine learning tools, and OCTA has shown great potential in identifying the most accurate biomarkers for glaucoma diagnosis.[27] Artificial intelligence and machine learning applications represent the future direction in glaucoma research and have the potential to increase the sensitivity and specificity of OCTA-derived vascular biomarkers for glaucoma diagnosis and elucidate other patterns of vascular abnormalities as they relate to regional defects in patients with ppOAG.

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