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Macular microvasculature alterations in patients with primary open-angle glaucoma

A cross-sectional study

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Abstract

To evaluate and compare macular microvasculature changes in eyes with primary open-angle glaucoma (POAG) to normal eyes, and to assess associations among the retinal microvasculature, neural structural damage, and visual field loss.

Ninety-nine eyes (68 patients with POAG and 31 normal subjects) were enrolled in this study. Thirty-five eyes with early-stage glaucoma (EG), 33 eyes with advanced-stage glaucoma (AG), and 31 normal eyes were included. An optical coherence tomography system with a split-spectrum amplitude-decorrelation angiography algorithm was used to measure the macular capillary vessel area density and retinal thickness. Visual field testing (30-2 and 10-2 programs) was performed using a Humphrey field analyzer. Correlations between the capillary vessel area density, retinal thickness, and visual field parameters were analyzed.

Compared to normal eyes, those with EG and AG had a lower macular capillary vessel area density and lesser retinal thickness ($P < 0.001$, all). Results of multivariate linear regression analyses showed that each standard deviation (SD) decrease in the vessel area density was associated with a 1.5% and 4.2% thinning of the full retinal thickness and inner retinal layer thickness, respectively. Each SD decrease in the vessel area density was also associated with a 12.9% decrease in the mean sensitivity and a 33.6% increase in the pattern standard deviation ($P < 0.001$, both). The Pearson partial regression analysis model showed that the vessel area density was most strongly associated with the inner retinal layer thickness and inferior hemimacular thickness. Furthermore, a lower vessel area density was strongly associated with a more severe hemimacular visual field defect and the corresponding hemimacular retinal thickness.

The macular capillary vessel area density and retinal thickness were significantly lower in eyes with POAG than in normal eyes. A diminished macular microvasculature network is closely associated with visual field defects, which are dependent on structural damage due to POAG.

Abbreviations: 3D = three-dimensional, AG = advanced-stage glaucoma, AL = axial length, BCVA = best-corrected visual acuity, C/D = cup-disc ratio, CCT = central corneal thickness, EG = early-stage glaucoma, FD-OCT = Fourier-domain optical coherence tomography, ILM = internal limiting membrane, IOP = intraocular pressure, IPL = inner-plexiform layer, MD = mean defect, MS = mean sensitivity, OCT = optical coherence tomography, ONH = optic nerve head, OPP = ocular perfusion pressure, POAG = primary open-angle glaucoma, PSD = pattern standard deviation, RGC = retinal ganglion cell, RNFL = retinal nerve fiber layer, RPE = retinal pigment epithelium, SD = standard deviation, SE = spherical equivalent, SSADA = split-spectrum amplitude-decorrelation angiography, VF = visual field.

Keywords: macular microvasculature, primary open-angle glaucoma, retinal thickness, visual field

1. Introduction

Primary open-angle glaucoma (POAG) is typically a chronic, multifactorial optic neuropathy characterized by progressive retinal ganglion cell (RGC) loss and visual field (VF) defects. Various baseline factors may affect the POAG disease processes, including an older age, higher intraocular pressure (IOP), larger cup-disc (C/D) ratio, greater vision defects, and lower corneal thickness.[1] The only current treatment for POAG is based on decreasing an increased IOP.[2,3] Nevertheless, a considerable proportion of patients with POAG have visual impairment, even when the IOP is significantly decreased following glaucoma surgery.[4,5]

Over the past few decades, increasing evidence has indicated that ocular blood flow and vessel autoregulation abnormalities play crucial roles in the etiology and progression of POAG. Several researchers have found various ocular hemodynamic deficits in eyes with POAG. These include decreases in the choroidal and retrobulbar blood flow in eyes with more advanced glaucoma[6,7] and abnormal retinal and choroidal vascular regulation in response to multiple types of provocations.[8,9]

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However, the mechanism by which retinal hemodynamic parameters change in eyes with POAG is somewhat controversial. Michelson et al[10] reported that the neuroretinal rim and retinal juxtapapillary blood flow, measured with scanning laser Doppler flowmetry, was markedly decreased in eyes with POAG compared to age-matched normal controls. In contrast, other investigators have found no difference in retinal peripapillary blood flow among normal eyes, eyes with POAG, and eyes with ocular hypertension.[11] Recently, Wang et al[12] reported a decrease in retinal blood flow in eyes with POAG using Doppler Fourier-domain optical coherence tomography (FD-OCT), a system known for its high scanning speed and good repeatability.[13]

Most studies have focused on abnormalities in large vessels or capillaries that supply the optic nerve head (ONH). Therefore, there is little evidence on macular hemodynamic changes in eyes with POAG. Liu et al[14] recently used optical coherence tomography (OCT) angiography combined with a split-spectrum amplitude-decorrelation angiography (SSADA) algorithm to quantify decreases in peripapillary retinal perfusion in eyes with glaucoma. The measured parameters included the flow index and vessel area density deficits, which both exhibited excellent repeatability and reproducibility. Therefore, the aims of the present study were to examine and compare changes in the macular microvasculature and retinal thickness in patients with POAG to normal subjects using SSADA-OCT, and to assess the associations among retinal microvascular changes, structural damage, and VF loss in the macular region in patients with POAG.

2. Methods

This research protocol was reviewed and approved by the Institutional Review Board of the Eye and ENT Hospital of Fudan University (No. 2014043, Shanghai, China). All subjects provided written informed consent to participate after the risks and benefits were explained to them. All study conduct adhered to the tenets of the Declaration of Helsinki.

2.1. Study subjects

Sixty-eight patients diagnosed as having POAG at the Eye and ENT Hospital of Fudan University and 31 age-matched normal subjects from July 2015 to June 2016 were recruited for this cross-sectional research study. Inclusion criteria for normal eyes were as follows: IOP <21 mm Hg, no presence of retinal retinopathy or glaucoma, no family history of glaucoma in a first-degree relative, a normal Humphrey Swedish Interactive Threshold Algorithm (SITA) 30-2 VF, and a normal C/D, retinal nerve fiber layer (RNFL), and ganglion cell complex (GCC) thickness. All included patients presented with the following conditions: glaucomatous optic nerve damage (e.g., diffuse neuroretinal rim thinning and localized notching, cup enlargement, or an OCT-documented RNFL defect), open anterior chamber angles in both eyes (confirmed via gonioscopy), and typical glaucomatous VF defects identified on at least 2 VF tests. Exclusion criteria were patients with any systemic disease, a history of intraocular surgery or trauma, any other disease known to contribute to VF defects or optic nerve damage, a refractive error more severe than −8.00 D or +3.00 D, unreliable VFs (i.e., fixation errors >20%, false positives >15%, or false negatives >33%), or a history of using ocular medications (topical eye drops for decreasing the IOP were acceptable for patient with POAG).

One eye from each subject was randomly selected as the study eye. Eyes with POAG were divided according to the Hodapp–Parrish–Anderson grading scale of VF defect severity, which was determined using the Humphrey SITA 30-2 VF testing protocol,[15] into an early-stage POAG (EG) group (mean defect [MD] > -6 dB) and an advanced-stage POAG (AG) group. The AG group included eyes with moderate ($-12\,\text{dB} < \text{MD} \leq -6\,\text{dB}$) and severe ($\text{MD} \leq -12$ dB) disease.

2.2. Study examinations

All subjects underwent ophthalmologic examinations, including the best-corrected visual acuity (BCVA) measurement, slit-lamp biomicroscopy, fundus examination, refractive error, and IOP measurement (Goldmann applanation tonometry). The IOL Master (Carl Zeiss Inc., Jena, Germany) was used to measure the central corneal thickness (CCT) and axial length (AL). The spherical equivalent (SE) was calculated as the spherical error plus one-half the cylindrical error, and this was used in the analyses. Subjects' heart rate and blood pressure (both systolic pressure and diastolic pressure) were also measured, and their medical and family histories were obtained. VF testing was performed using a Humphrey field analyzer (SITA full-threshold programs 30-2 and 10-2; Carl Zeiss Meditec, Inc., Dublin, CA).

2.3. OCT image acquisition and processing

All OCT angiography scans were collected using the spectral-domain system (RTVue-XR Avanti, Optovue Inc., Fremont, CA). The instrument has a light source centered at 840 nm, bandwidth of 45 nm, and an A-scan speed of up to 70 kHz. Three-dimensional (3D) OCT angiography scans were composed of 5 consecutive B-scans at 216 raster positions that covered a 6.0×6.0-mm macular region. Each B-scan consisted of 216 A-scans. Two volumetric raster scans containing a horizontal priority (x-fast) and a vertical priority (y-fast) were acquired consecutively. Motion artifacts were removed using 3D orthogonal registration and 2D scan merging. All en face retinal angiograms were generated using flow signal projection from the internal limiting membrane (ILM) to the retinal pigment epithelium (RPE) using RTVue-XR Avanti software.

2.4. Measuring the macular microvasculature index

Macular scans covered a 6.0×6.0-mm area, in which the microvasculature vessel area density was measured. The macular vessel area density was defined as the fractional area occupied by flow pixels[16] within an annulus that had an inner diameter of 1 mm and an outer diameter of 5 mm.

2.5. Retinal thickness analyses

Retinal thickness measurements in each area were automatically obtained and calculated using the retinal map protocol in the Avanti RTVue-XR software. Using an OCT angiogram, parafoveal thickness was measured within a circular annulus (inner radius = 1.00 mm, outer radius = 3.00 mm) centered on the fovea. The perifoveal area was defined as a 2-mm-wide circular annulus extending from the parafoveal boundary.

The full retinal layer thickness was defined by the algorithm as the distance between the ILM and the middle of the RPE. The inner retinal layer thickness was defined by the algorithm as the distance between the ILM and the outer boundary of the inner-plexiform layer (IPL). The average and regional information (superior

Table 1

Baseline characteristics of the normal subjects and patients with glaucoma.

	Normal group	Early-stage POAG group	P*	Advanced-stage POAG group	P†
Age, y	39±13	42±14	0.516	42±13	0.532
Sex ratio, male:female	11:20	11:24	0.727	26:7	<0.001‡
C/D ratio	0.51±0.12	0.77±0.15	<0.001§	0.88±0.10	<0.001§
BCVA	1.1±0.3	0.9±0.3	0.082	0.8±0.3	<0.001§
SE (D)	−2.2±2.4	−2.9±2.9	0.336	−3.5±3.6	0.091
AL, mm	24.9±1.4	25.2±1.7	0.536	25.53±2.00	0.143
CCT, μm	540.2±29.3	544.1±20.0	0.615	525±42	0.059
IOP, mm Hg	14.3±2.5	15.9±4.1	0.089	16.2±4.7	0.051
OPP, mm Hg	42.2±8.3	45.3±8.0	0.109	45.23±7.05	0.128

Results are presented as the mean±standard deviation.

AL=axial length, BCVA=best corrected visual acuity, C/D=cup-disk, CCT=central corneal thickness, IOP=intraocular pressure, OPP=ocular perfusion pressure, POAG = primary open-angle glaucoma, SE (D)=spherical equivalent (diopter).

* Normal vs early-stage POAG.

† Normal vs advanced-stage POAG.

‡ $P<0.01$, tested with 1-way analysis of variance.

§ $P<0.01$, tested with the chi-squared test.

hemisphere and inferior hemisphere) was obtained directly from this system. In our study, macular retinal thickness, including the full and inner macular thicknesses, was defined as the average value in the parafoveal and perifoveal areas.

2.6. Correlating retinal structure and function

The VF indexes, which included the pattern standard deviation (PSD), mean sensitivity (MS), and MD, were obtained using the 10-2 SITA Standard program of the Humphrey VF analyzer. In the SITA Standard 10-2 program, the global central MS is calculated as the average of 68 VF points within a 4.8-mm circular area centered on the topographical fovea. The superior hemimacular MS was calculated from the average MS values of the 34 test points within the superior hemifield, and it corresponded with the inferior hemimacular retinal thickness measured using OCT angiography. Likewise, the inferior hemimacular MS was calculated from the average MS value of 34 test points within the inferior hemifield, and it corresponded with the superior hemimacular retinal thickness. The MS magnitude reflected the severity of VF damage. The more severe hemimacular VF defect was selected for each eye and included in the hemi-MS-severe group. The second hemimacular VF defect was identified and included in the hemi-MS-mild group. The hemimacular full and inner retinal thickness measurements that corresponded to each VF defect were categorized in the hemi-full-severe, hemi-full-mild, hemi-inner-severe, and hemi-inner-mild groups.

2.7. Statistical analyses

Statistical analyses were performed using SPSS statistical software (version 20.0, SPSS, Inc., Chicago, IL). Differences between the normal group and glaucoma groups in terms of the macular vessel area density, macular retina thickness, and several basic characteristics were first compared using 1-way analysis of variance. After adjusting for age, sex, BCVA, SE, IOP, CCT, AL, C/D, and ocular perfusion pressure (OPP) of patients with POAG, a multivariate linear regression analysis was used to examine correlations between macular microvasculature changes and structural damage. This same analysis was also used to assess correlations between macular microvasculature changes and 10-2 SITA Standard VF defects. The OPP was calculated from the mean arterial pressure using the following equations:

$$\mathrm{MAP} = \frac{1}{3}(2 \times \mathrm{DP} - \mathrm{SP})$$

$$\mathrm{OPP} = \frac{2}{3}(\mathrm{MAP} - \mathrm{IOP})$$

Pearson partial correlation analyses were used to compare the strengths of the correlations. The macular vessel area density was defined as the independent variable, and the structural or VF parameters were defined as dependent variables in all regression analyses. Statistical significance was defined as $P<0.05$. All P values were 2-sided.

3. Results

3.1. General information of participates

Ninety-nine eyes of 99 subjects (68 patients with glaucoma and 31 age-matched normal subjects) were included in this study. According to the glaucomatous grading system, 35 eyes were categorized in the EG group and 33 eyes were categorized in the AG group (11 eyes with moderate disease and 22 eyes with advanced disease). Subjects' characteristics are summarized in Table 1.

3.2. Changes in the macular microvasculature and retinal thickness

Table 2 presents an overview of the data of the macular microvasculature and retinal thickness among the 3 groups. The

Table 2

Mean macular microvasculature and retinal thickness for normal, early-stage, and advanced-stage POAG patients.

	Normal	EG*	AG†,‡
Vessel area density, %	76.5±3.9	70.2±6.3	60.1±7.5
Full retina thickness, μm	304.0±11.2	291.3±13.7	267.9±20.9
Inner retina thickness, μm	123.0±6.8	111.3±9.1	95.8±12.2

AG=advanced-stage glaucomatous patients, EG=early-stage glaucomatous patients, POAG = primary open-angle glaucoma.

* $P<0.001$ for all (normal vs EG).

† $P<0.001$ for all (normal vs AG).

‡ $P<0.001$ for all (EG vs AG).

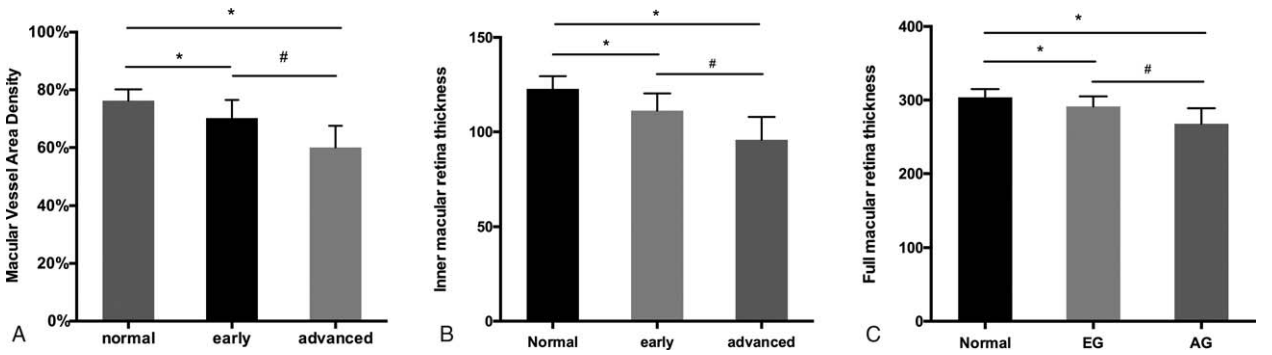

Figure 1. Comparisons of the macular vessel area density (A), inner-macular retina thickness (B), and full-macular retina thickness (C) among normal eyes, eyes with early-stage, and advanced-stage primary open-angle glaucoma. Statistical significance was calculated using 1-way analysis of variance. Results are presented as the mean ± standard deviation. $^{*}P < 0.01$, normal vs early-stage glaucoma (EG) and advanced-stage glaucoma (AG). $^{\#}P < 0.01$, EG vs AG.

mean macular vessel area density was significantly lower in the EG group than in the normal group ($P < 0.001$; Fig. 1A). The mean inner and full retinal thicknesses were also thinner in the EG group than in the normal group ($P < 0.001$, both; Fig. 1B and C). Furthermore, there were significant differences in the macular vessel area density and full and inner retinal thicknesses between the EG and AG groups ($P < 0.001$, all; Fig. 1, Table 2).

3.3. Mapping macular microvasculature changes to structural damage and VF defects

Macular OCT retinal angiograms, retinal map images, and 10-2 SITA Standard VF testing results for the normal, EG, and AG groups are shown in Fig. 2. A dense and symmetrical distribution of the capillary network in the macular region was visible in the normal group (Fig. 2A). However, compared to the normal group, the EG group had focal capillary network dropout, especially in the inferotemporal macular region (Fig. 2D). In the AG group, only a sparse microvascular network was visible on angiograms, and capillaries were present in a much lower number than the normal group throughout the entire macular area (Fig. 2G). Additionally, retinal thickness maps of the EG group showed focal macular thinning in the inferior and temporal regions, which was indicated by the full retinal thickness measurements. These areas of thinning corresponded with the locations of macular microvasculature defects (Fig. 2E). Moreover, all regional retinal thickness measurements were lower in the AG group than in the normal group, which matched the pattern of an attenuated capillary network over the entire macula (Fig. 2H). Moreover, SITA Standard 10-2 VF defects on all PSD maps corresponded with the locations of macular capillary dropout and retinal thinning (Fig. 2C, F, I).

3.4. Correlations between the macular microvasculature, structure, and visual function

Firstly, correlations between the variables could be observed intuitively from Fig. 3. Results of multiple linear regression analysis showed a positive correlation between the macular vessel area density and inner or full retinal macular thickness ($P < 0.001$, both; Table 3). In addition, a decrease in the vessel area density by 1 standard deviation (SD) indicated 1.5% thinning of the full retina thickness and 4.2% thinning of the inner retinal layer thickness. The MS was positively correlated with the vessel area density ($P < 0.001$), the group male ratio ($P = 0.04$), and C/D ($P = 0.02$) (Table 3). The PSD was negatively correlated with the macular vessel area density ($P < 0.001$, Table 3). A decrease in the vessel area density of 1 SD indicated a 12.9% decrease in MS and a 33.6% increase in PSD.

3.5. Comparisons of correlation strengths

On the basis of data obtained by Pearson partial regression analyses, we found that the vessel area density was more strongly correlated with the inner macular thickness than the full macular thickness. Furthermore, results of a hemimacular analysis showed the strongest correlation between the macular vessel area density and inferior hemimacular thickness (Table 4). Additionally, findings regarding the microvasculature-visual function relationship indicated that the vessel area density was more strongly associated with the hemi-MS-severe group than with the hemi-MS-mild group (Table 5). Likewise, the correlation strength between the vessel area density and hemi-full-severe/hemi-inner-severe thickness values was greater than that between the vessel area density and hemi-full-mild/hemi-inner-mild thickness values (Table 5).

4. Discussion

Our study's results indicated that eyes with EG and AG had a lower macular capillary vessel area density and a thinner retina than normal eyes. Furthermore, in the macular region, a significant positive correlation existed between microvasculature deficits and retinal layer damage, and visual function defects. These correlations were identified after adjusting for potential confounding factors. Additionally, results of a detailed regional analysis showed an obvious decrease in the capillary vessel area density, which was strongly associated with inferior hemimacular retinal thinning and more severe hemimacular VF loss.

Almost 50% of RGCs are distributed in the macula.[17] Studies in primates have shown that the RGC density reaches a maximum within the foveal slope, approximately 0.5 mm from the foveal pit.[18] Postmortem studies on human eyes with glaucoma have shown that eyes with no VF damage have a >50% loss of RGCs.[19] The retina and especially the macula consume more oxygen per weight than any other tissue in the mammalian body[20]; thus, the macula is likely susceptible to hypoxic and ischemic damage.[21] Additionally, many macular

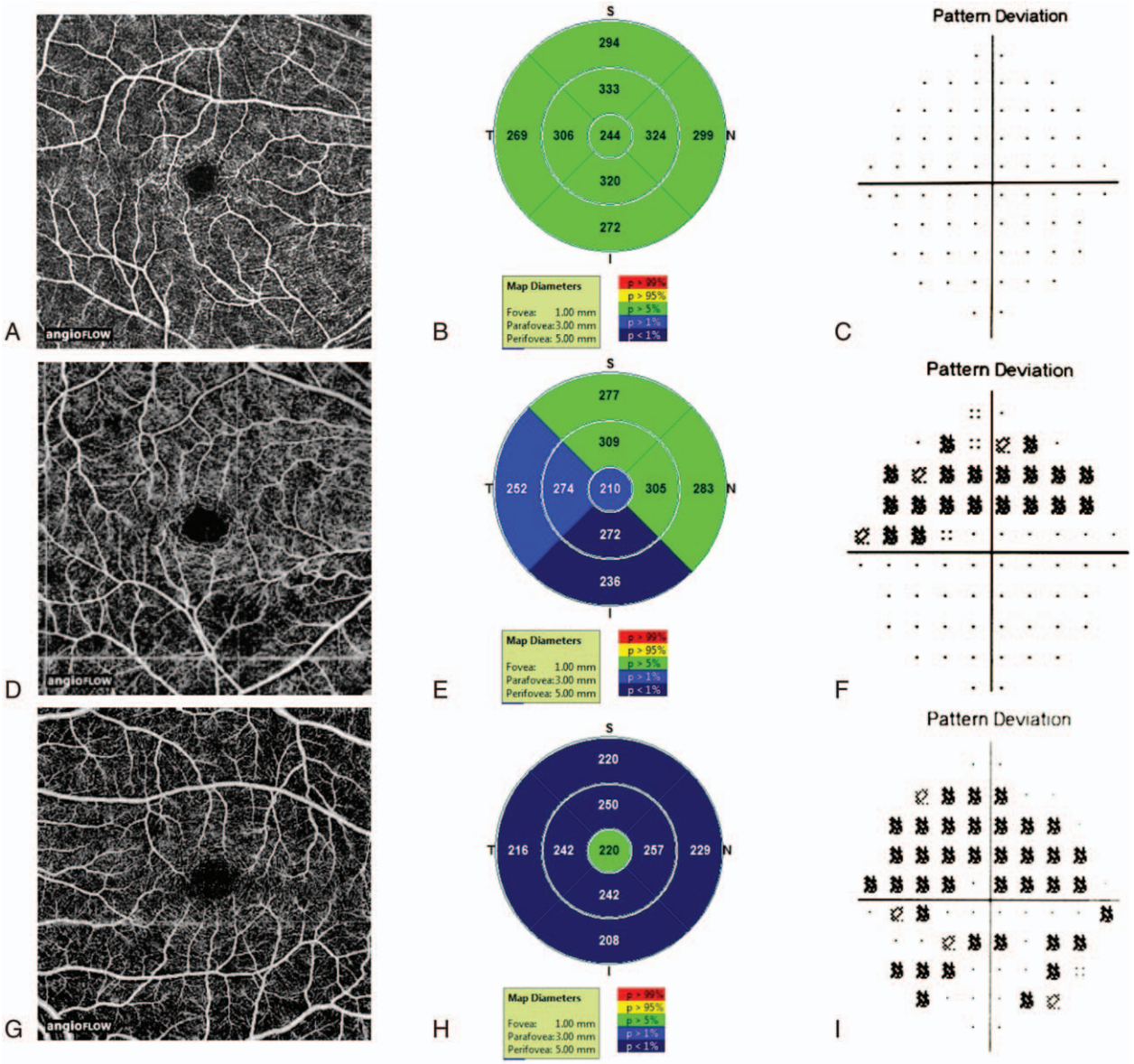

Figure 2. Mappings of the microvasculature (A, D, G), structure (B, E, H), and visual function (C, F, I) in normal eyes (A, B, C), eyes with early-stage (D, E, F), and advanced-stage primary open-angle glaucoma (G, H, I).

vascular diseases have pathological changes that primarily begin at the capillary level. Additionally, these vessels supply a significant proportion of the ganglion cells, which play a crucial role in the etiology and progression of POAG. Therefore, we thought it was important to investigate whether retinal microvascular abnormalities occur in the macular region in patients with POAG.

We found that the macular capillary vessel area density was lower in eyes with EG and AG than in normal eyes. Additionally, focal or symmetrical capillary network dropout in glaucomatous eyes was visible on retinal angiograms. These results are in agreement with findings of Liu et al's study,[14] which showed that glaucomatous eyes had decreased peripapillary retinal perfusion on OCT angiograms (the same OCT system used in the present study). Our findings are also supported by the results of Wang's study,[13] which showed decreased retinal perfusion in glaucomatous eyes, as measured with Doppler FD-OCT. However, these 2 studies mainly concentrated on hemodynamic parameters of the vessels that supply the ONH. In contrast, Burgansky-Eliash et al[22] found no significant difference in the macular hemodynamic parameters, including the arterial and venous velocities, between eyes with EG and AG. Their measurements were made using a retinal function imager, which is limited to measuring blood velocity in medium-sized vessels. This may explain why their results differed from ours, because glaucomatous vascular impairment may begin in the capillary network. The OCT angiography technique used in our study, which has a high repeatability and reproducibility when measuring peripapillary perfusion,[14] was mainly used to examine the microvasculature in the macular region, as it provides detailed images.

Our data showed that the inner and full retinal layer thicknesses were significantly and positively correlated with the retinal capillary vessel area density. Previous studies have

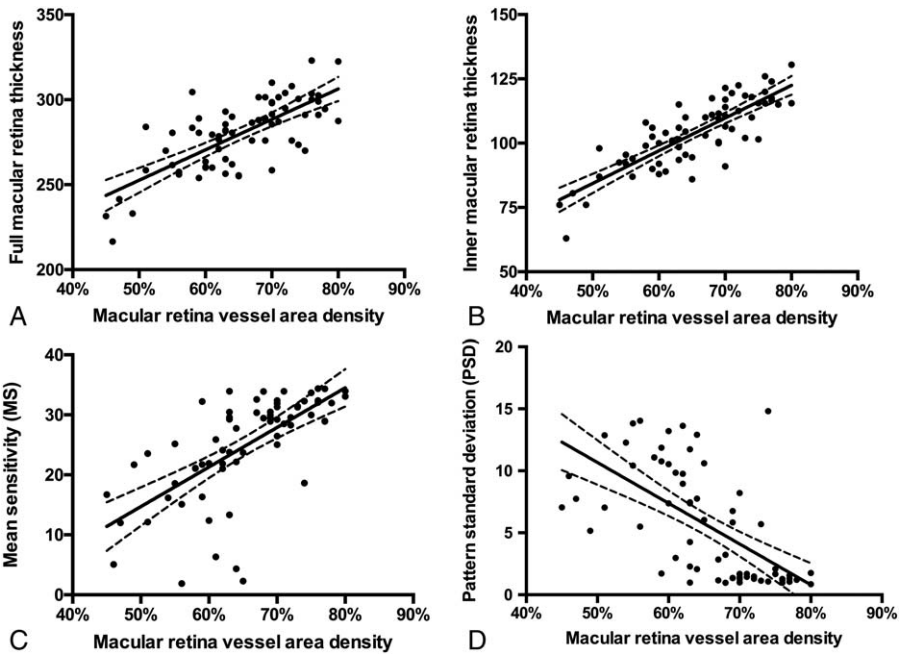

Figure 3. Correlations between the macular retinal vessel area density and retinal thickness or visual field parameters. (A) Macular retinal vessel area density and full macular retina thickness, (B) inner macular retina thickness, (C) mean sensitivity, and (D) pattern standard deviation. The 95% confidence interval is indicated by a dashed line.

investigated the relationship between ocular blood flow changes and structural damage in eyes with glaucoma, but the results have varied. Using the same technique as used herein, another study by our group[23] showed a close correlation between optic disc microvasculature perfusion and RNFL, and GCC thickness in eyes with POAG. Similar to our findings, Tobe et al[24] found that optic disc rim thinning was associated with lower retinal and retrobulbar blood flow, as measured with confocal laser Doppler and color Doppler imaging, respectively. In contrast, Berisha et al[25] observed an inverse correlation between the RNFL thickness and blood flow reduction in EG eyes, as measured using a Canon laser Doppler retinal blood flow instrument. Although the underlying cause of these differences has not been determined, varying hemodynamic parameter measurement techniques and the examination of different ocular regions and tissues could explain it to some extent. In the study by Berisha et al,[25] only 1 length of a given retinal artery that was adjacent to the optic disc was selected for hemodynamic parameter measurement. The technique used in our study had the advantage of providing data that is representative of the entire retinal microcirculatory network in the measurement area. The capillary vessel area density is an average measurement that reflects the perfusion status of the entire tissue/region.

The macular vessel area density was defined as the independent variable in regression analyses, and it was more strongly correlated with the inner retinal layer thickness than with the

Table 3

Multivariable correlational model of the macular vessel area density, macular thickness, and visual field parameters of patients with POAG.

	Full-macular thickness		Inner-macular thickness		MS		PSD	
Variables	**ß**	***P***	**ß**	***P***	**ß**	***P***	**ß**	***P***
Vessel area density	0.57	<0.001**	0.65	<0.001**	0.52	<0.001**	−0.59	<0.001**
Age, y	0.00	0.99	−0.05	0.50	−0.12	0.24	0.02	0.89
Sex	−0.04	0.72	−0.04	0.64	0.24	0.04*	0.09	0.46
C/D ratio	−0.10	0.31	−0.15	0.04*	−0.23	0.02*	0.31	0.01**
BCVA	0.14	0.15	0.15	0.05	0.15	0.12	−0.00	>0.99
SE (D)	−0.06	0.72	0.07	0.58	−0.12	0.46	−0.01	0.97
AL, mm	−0.27	0.09	−0.18	0.13	0.18	0.25	−0.15	0.39
CCT, μm	0.10	0.39	0.11	0.18	0.13	0.24	−0.028	0.82
IOP, mm Hg	−0.01	0.94	0.01	0.93	−0.01	0.92	0.15	0.25
OPP, mm Hg	0.01	0.95	0.04	0.63	0.11	0.34	0.13	0.30

The ß value is a measure of how strongly the independent variable (first column) affects the dependent variable (first row), and it is measured in standard deviation units.

AL = axial length, BCVA = best corrected visual acuity, C/D = cup-disk, CCT = central corneal thickness, IOP = intraocular pressure, MS = mean sensitivity, OPP = ocular perfusion pressure, POAG = primary open-angle glaucoma, PSD = pattern standard deviation, SE (D) = spherical equivalent (diopter).

* $P < 0.05$.

** $P < 0.01$, calculated from multivariable linear regression analysis.

Table 4

Pearson partial correlation coefficients between the macular vessel area density and macular structural measurements.

	Full macular		Full superior		Full inferior	
	$\|r_1\|$	*P*	$\|r_2\|$	*P*	$\|r_3\|$	*P*
Vessel area density	0.54	<0.001*	0.41	<0.01*	0.62	<0.001*
	Inner macular		**Inner superior**		**Inner inferior**	
	$\|r_4\|$	*P*	$\|r_5\|$	*P*	$\|r_6\|$	*P*
Vessel area density	0.69	<0.001*	0.57	<0.001*	0.72	<0.001*

|r| = Pearson partial correlation coefficient, Full inferior = full retina thickness of the inferior hemimacular, Full macular = full retina thickness of the macular region, Full superior = full retina thickness of the superior hemimacular, Inner inferior = inner retina thickness of the inferior hemimacular, Inner macular = inner retina thickness of the macular region, Inner superior = inner retina thickness of the superior hemimacular.

* $P < 0.01$.

full retinal layer thickness. In our study, the inner retinal layer was defined as the ILM, RNFL, RGC, and IPL. One possible explanation for these results was that most RGCs resided in the inner retinal layers, which completely obtain their oxygen supply from the superficial retinal capillary plexus. In contrast, the outer one-third of the retina has oxygen and nutrients supplied by choroidal circulation.[26] Therefore, the inner retinal layer, especially the RGC is more susceptible to damage from decreased retinal perfusion than the retina as a whole. In the present study, our data suggest that ganglion cell loss may partly result from an attenuated retinal microcirculation network in eyes with POAG.

Results of regional analyses showed that the macular capillary vessel area density was more strongly correlated with inferior hemimacular structural damage than with superior hemimacular structural damage. This finding indicates that the inferior hemimacular retinal structure is susceptible to a decrease in the retinal capillary vessel area density in glaucomatous eyes. Our results confirm the observation by Leung[27]; only inferior arcuate fiber thickness in the macular region differed between normal eyes and those considered to have glaucoma. Furthermore, our results concur with previous findings on inferior and temporal ONH vulnerability to early glaucomatous optic nerve atrophy.[28,29] Tobe et al[24] found a significant increase in the area of nonperfused retina, particularly in the inferior region, over 18 months in eyes with POAG. These findings suggest that retinal microvasculature abnormalities and structural damage occur more easily in the inferior areas of the retina.

Results of multivariate linear regression analyses showed that decreases in the vessel area density were associated with increases in VF loss. Hwang et al[30] found that VF loss was correlated with decreased retinal blood flow in a region centered on the optic disc. These findings were consistent with those of Liu's study,[14] which showed that VF indexes were correlated with the retinal peripapillary flow index and vessel area density. In the present study, Pearson partial regression analyses among macular capillary vessel area density and hemimacular structural, and VF damage indicate that VF loss may result from blood flow changes mediated by structural damage. However, double circular Doppler OCT images from a previous study demonstrated that diminished blood flow was associated with VF defects, independent of RNFL or disc rim thinning.[30] This difference in results may have been caused by inherent differences in the retinal blood flow measurement techniques. Double circular Doppler OCT does not assess localized retinal blood flow or the microcirculation that supplies the retina. Our method has the advantage of being able to detect subtle changes in the local region, and microcirculatory parameters may be more sensitive than large vessel hemodynamic indexes to changes causing or resulting from glaucomatous damage.

Our study has some limitations. As this was not a longitudinal study, changes over time in each subject could not be evaluated.

Table 5

Pearson partial correlation coefficients between the macular vessel area density and hemimacular visual field defects, and corresponding structural measurements.

	Hemi-MS-severe		Hemi-MS-mild	
	$\|r_1\|$	*P*	$\|r_2\|$	*P*
Vessel area density	0.52	<0.001*	0.40	<0.01*
	Hemi-full-severe		**Hemi-full-mild**	
	$\|r_3\|$	*P*	$\|r_4\|$	*P*
Vessel area density	0.61	<0.001*	0.42	<0.01*
	Hemi-inner-severe		**Hemi-inner-mild**	
	$\|r_5\|$	*P*	$\|r_6\|$	*P*
Vessel area density	0.71	<0.001*	0.61	<0.001*

Hemi-full-mild = full hemimacular thickness corresponding with the hemi-MS-mild, Hemi-full-severe = full hemimacular thickness corresponding with the hemi-MS-severe, Hemi-inner-mild = inner hemimacular thickness corresponding with the hemi-MS-mild, Hemi-inner-severe = inner hemimacular thickness corresponding with the hemi-MS-severe, Hemi-MS-mild = the milder hemimacular visual field defect (mean sensitivity), Hemi-MS-severe = the severer hemimacular visual field defect (mean sensitivity).

|r| = Pearson partial correlation coefficients.

* $P < 0.01$.

Therefore, we cannot conclude whether macular microvasculature changes cause or result from degenerative glaucomatous processes. Additionally, unlike retinal thickness map data, the macular capillary vessel area density cannot be divided into hemispherical or regional measurements. Therefore, we could not assess a focal correspondence among capillary network defects, structural damage, and VF loss. Future studies are needed to examine the capillary vessel area density in each retinal quadrant, and further assess correlations between the microvasculature, retinal structure, and visual function in various regions. Furthermore, following up with the same group of patients over the long term may enable physicians to better understand how and why microvasculature changes occur with glaucoma progression, which will build on our study's findings.

In conclusion, significant differences between eyes with glaucoma and normal eyes in terms of the macular retinal thickness and microvasculature suggest that both retinal thickness and the microvasculature are involved in the etiology and progression of glaucoma. Therefore, the retinal capillary vessel area density may be a reliable indicator of EG. Additionally, our study's findings showed that a decrease in the macular microvasculature is associated with VF defects, which are dependent upon structural damage in eyes with POAG.

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