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ORIGINAL PAPER

Examination of retinal vascular density changes via optical coherence tomography angiography in patients with glaucoma

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Abstract

Purpose The aim of this study was to investigate the changes in vessel density (VD) in glaucoma patients and to investigate the relationship between VD and structural parameters using optical coherence tomography angiography.

Methods Our study included 25 primary open-angle glaucoma (POAG) patients, 25 pseudoexfoliation glaucoma (PXG) patients and 25 healthy individuals. All patients underwent 6 × 6 mm retinal angiography (upper limit: internal limitan membrane [ILM], lower limit: 10 µm inferior to inner plexiform layer) and 4.5 × 4.5 mm disk angiography (upper limit: ILM, lower limit: posterior border of the retinal nerve fiber layer [RNFL]) via an optical coherence tomography (OCT) system (AngioVue, Optovue). Measures of vascular density were as follows: total area VD (tVD), intrapapillary VD (iVD), peripapillary VD (pVD) and parafoveal VD (pfVD). In addition to performing comparisons, the correlations between pVD, retinal nerve fiber layer (RNFL) thickness and optic nerve head measurement results were investigated.

Results A total of 75 individuals were included in our study. In the POAG and PXG groups, tVD and pVD values were significantly lower than the control group (median tVDs were: 45.4, 45.9, 50.0, and median pVDs were: 50.0, 50.3, 53.1, respectively) (confidence intervals were: − 9.8/− 1.1 for pVD and − 8.6/− 1.4 for tVD). Significantly lower pf-VD values were detected in the PXG group compared to the control group ($p < 0.05$). There were strong positive correlations between RNFL thickness and pVD in in glaucoma groups ($p < 0.001$). There was no correlation between pVD and disk area, intraocular pressure and age in glaucoma patients. Patients using beta-blockers had significantly lower tVD and pVD values compared to those who did not ($p < 0.05$).

Conclusion The low VD values in glaucomatous eyes and the strong correlations between that values and RNFL thickness demonstrate a relationship between structural parameters and vascular parameters.

Keywords Optical coherence tomography angiography · Vessel density · Primary open-angle glaucoma · Pseudoexfoliation glaucoma

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Introduction

Glaucoma is one of the leading causes of irreversible blindness worldwide [1]. There are studies in the literature showing the role of impaired ocular blood flow in glaucoma pathogenesis and these studies suggest that deterioration of blood flow to the optic nerve head (ONH) is not only a result of glaucomatous damage, but an early finding of the process [2–4]. However, the measurement of ONH blood flow has not become a part of standard examination in the diagnosis and follow-up of glaucoma due to unreliability, potential side effects and impracticality of routine use.

Similarly, impaired blood flow at the macula level is also important in glaucoma pathogenesis. It is believed that the death of retinal ganglion cells (RGCs), which ultimately has a central role in glaucoma pathogenesis, is associated with insufficient blood flow in the capillary network supplying RGCs which have high metabolic needs [5].

Optical coherence tomography angiography (OCTA) is a noninvasive imaging method that detects blood flow from the contrast of erythrocyte motions in blood vessels [6]. The OCTA modality has potential to bring innovations to the diagnosis and follow-up of glaucoma due to its qualitative and quantitative evaluation of the microvascular structure.

In this study, it was aimed to compare the vessel density (VD) parameters of glaucoma patients and normal cases by using OCTA and to examine the possible relationships between VD values and RNFL thickness.

Materials and methods

A total of 75 eyes, including 25 primary open-angle glaucoma (POAG) patients, 25 pseudoexfoliation glaucoma (PXG) patients and 25 healthy individuals, were included in the study. The study was planned as a prospective, single-center study. Ethics committee approval and consent of the patients were obtained before the study.

Criteria for inclusion into the study were determined as follows: being between the ages of 40–80 years, having no history of ocular surgery except for cataract surgery (which should not have been performed within the last 6 months), having a best corrected visual acuity of 0.5 and above with standart Snellen chart measures, the absence of medium opacities such as corneal opacity or vitreous opacity, having spherical or cylindrical refractive error values that did not exceed + 3 or − 3 diopters. Additionally, people with systemic diseases that may affect the vascular structure such as diabetes mellitus (DM), hypertension (HT) and coronary artery disease and obstructive sleep apnea syndrome and those using systemic drugs that may affect vascular diameter were excluded from the study. In addition to these criteria, patients with glaucoma had to have been diagnosed with POAG or PXG based on intraocular pressure (IOP), biomicroscopic examination, optic disk and visual field findings, and they were excluded if they had previously undergone glaucoma surgery or had any eye disease other than glaucoma and cataract. For the healthy group, individuals that had normal ophthalmological examination and did not have a family history for glaucoma were included.

The best corrected visual acuity (BCVA) was measured using the Snellen chart and recorded as a decimal value. IOPs and central corneal thicknesses (CCT) were measured. After anterior segment examination, topical eye drops of tropicamide 0.5% were applied for pupil dilation. Phenylephrine drops were avoided when obtaining pupil dilation.

Following a 15-min resting period after pupil dilatation, we examined all patients using the AngioVue (RTVue-XR, Fremont, California, USA) software (version 2017.1.0.151) which is a dual modal OCT system that can provide SD-OCT and OCTA examination and performs structural and vascular measurements together. Macular OCT, 6 × 6 mm retinal angiography and centered ONH 4.5 × 4.5 mm disk angiography were obtained in each patient. Central macular thickness (CMT), RNFL analysis, ONH analysis, parafoveal and disk VD results were obtained and recorded. The same protocol was executed for the control group.

Measurement was done with a 6 × 6 mm scan for parafoveal VD evaluation. Superficial plexus VD values were determined automatically with the software. In order to evaluate the superficial plexus, the upper limit was determined as internal limiting membrane (ILM), and the lower limit was determined 10 μm below the inner plexiform layer. The superficial layer VDs of the temporal, inferior, nasal and superior quadrants of the parafoveal (pf) ring and the

mean parafoveal VD were used as study parameters (Fig. 1). To examine the radial peripapillary capillary (RPC) vascularity at the optic disk head, the borders were determined as ILM and the posterior edge of the retinal nerve fiber layer. The values for the total area VD (tVD), intrapapillary VD (iVD) and peripapillary VD (pVD) were determined, and the pVD superior/inferior halves as well as pVD superior–inferior–nasal–temporal quadrants were examined (Fig. 2). Images with signal strength index values lesser than 6/10 and those with image artifacts due to movement were excluded from the study.

Statistical analysis

Descriptive statistics are given as mean ± standard deviation (SD) for continuous variables that fit normal distribution, median and 25–75 percentile values for numerical variables that did not fit normal distribution. Categorical variables were presented with numbers and percentages. It was determined by the Shapiro–Wilk test whether the variables obtained fit normal distribution. One-way analysis of variance and post-hoc Bonferroni tests were used to compare data with normal distribution among the three groups (PXG, POAG and control groups), whereas the Kruskal Wallis test was used to compare variables that did not fit normal distribution between three groups. The Mann–Whitney *U* test was used in binary group comparisons that did not conform to the normal distribution. Since the average age of the PXG group was significantly higher than that of the POAG group and the controls, general linear model-univariate analysis was used, taking age as a cofactor in all three groups. Chi-square tests were used for comparisons between categorical variables. Pearson or Spearman correlation analyses were used to determine correlations between continuous variables. Since the average age of the POAG and PXG groups was significantly different and age was correlated with many variables in the control group, we applied partial correlation analyses by clearing the age effect when measuring correlations in the patient groups and control group. The PASW Statistics (version 18.0) software was used for statistical analysis, and all $p < 0.05$ values were considered to show statistical significance.

Results

The study included 75 eyes of 75 individuals, 25 of whom had POAG, 25 had PXG and 25 were controls. Of the 75 individuals included in the study, 32 were female, and 43 were male. There was no statistically significant difference between the groups in terms of gender distribution ($p > 0.05$) (Table 1). In terms of age averages, there was no significant difference between the POAG group and the control group ($p > 0.05$), whereas the average age of the PXG group was found to be significantly higher compared to other groups ($p < 0.05$) (Table 1).

There was no significant difference between the groups in terms of intraocular pressure and CCT measurement results ($p > 0.05$). In terms of cup/disk value, the values of both glaucoma groups were significantly higher than the control group ($p > 0.05$). There was no significant difference between the groups in the disk area analysis ($p > 0.05$). While there was no difference between glaucoma groups in terms of rim area and mean RNFL thickness

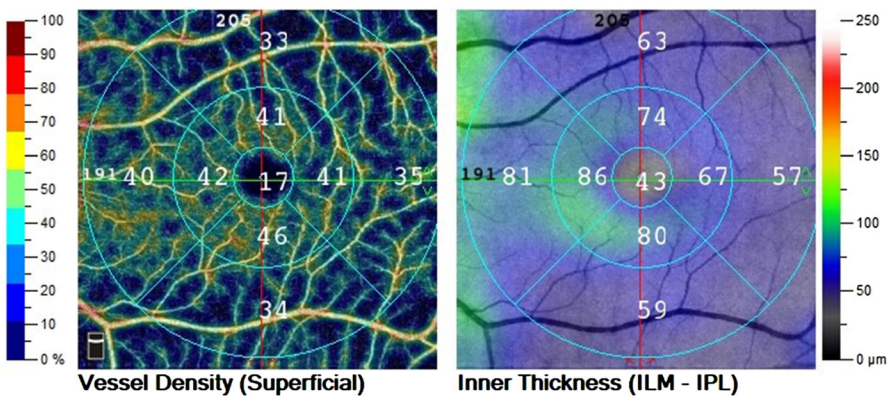

Fig. 1 Example of a 6 × 6 mm retinal angiography scan in the primary open-angle glaucoma group

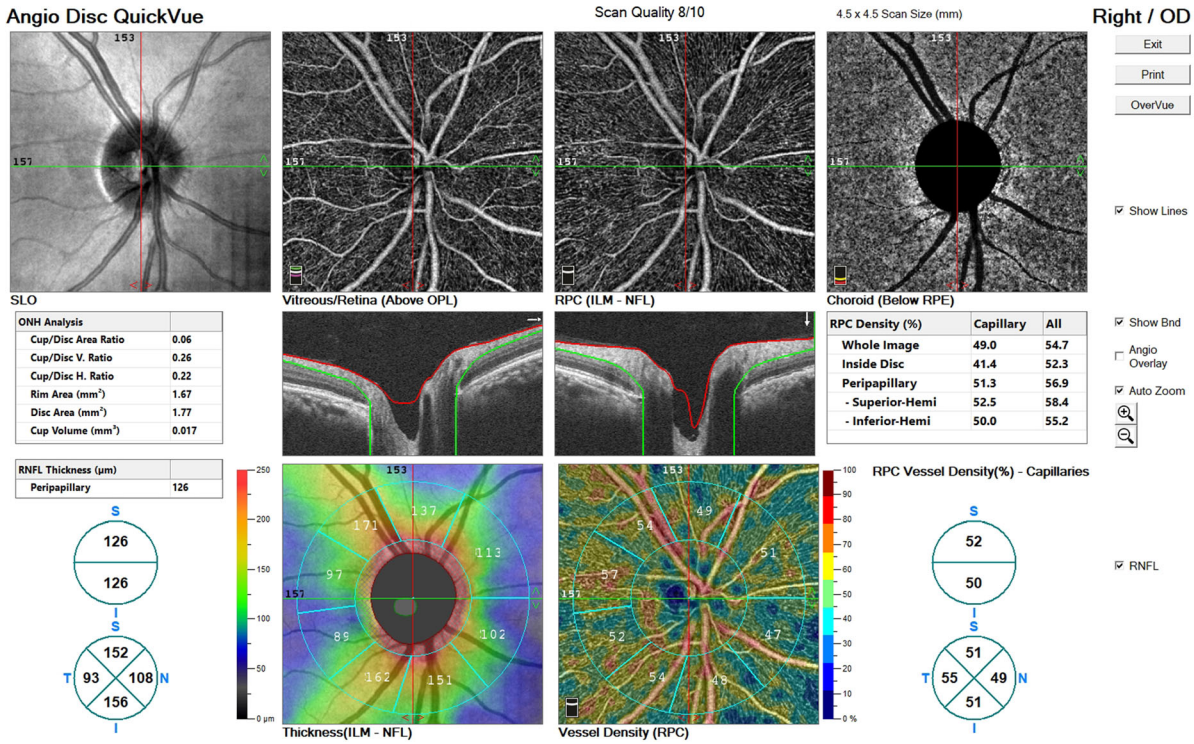

Fig. 2 Example of a 4.5 × 4.5 mm disk scan from the healthy control group

Table 1 Demographic and ocular characteristics of the study population

	Groups			*p* value		
	POAG[1] (*n* = 25)	PXG[2] (*n* = 25)	Control[3] (*n* = 25)	p^{1-2}	p^{1-3}	p^{2-3}
Female/male	12/13	10/15	10/15	0.56	0.56	1
Age, Year ‡	59.4 ± 6.9	68.7 ± 7.7	58.2 ± 5.0	**< 0.001**	1.000	**< 0.001**
BCVA*	0.9 (0.7–1.0)	0.9 (0.5–1.0)	1.0 (1.0–1.0)			
IOP, mmHg*	16 (15–17)	16 (14–17)	15 (14–16)	1.000	0.282	0.576
CCT, µm*	545 (526–570)	555 (530–584)	564 (560–574)	0.340	0.069	0.742
cup/disk ratio*	0.6 (0.4–0.7)	0.4 (0.4–0.6)	0.2 (0.1–0.3)	0.258	**< 0.001**	**0.002**
pseudophakic (*n*)	2	8	2			
Rim area, mm^2 ‡	1.41 ± 0.39	1.45 ± 0.30	1.71 ± 0.31	0.445	**0.002**	**0.017**
Disk area, mm^2 ‡	2.08 ± 0.25	1.94 ± 0.28	2.03 ± 0.29	0.147	1.000	0.613
RNFL-avg, µm ‡	87 ± 20	94 ± 20	115 ± 11	0.365	**< 0.001**	**< 0.001**

Statements in bold indicate statistically significant results

BCVA best corrected visual acuity, *CCT* central corneal thickness, *IOP* ıntraocular pressure, *RNFL* retinal nerve fiber layer thickness

‡Values are shown as mean ± standard deviation

*Values are shown as medians (25–75%)

($p > 0.05$), they were found to be significantly lower in the two glaucoma groups compared to healthy controls ($p < 0.05$) (Table 1).

While there was no significant difference between the glaucoma groups in terms of tVD values ($p = 1.000$), the tVD values of both POAG and PXG groups were significantly lower than the control group (median tVDs were: 45.4, 45.9, 50.0, respectively, confidence interval [CI]: − 9.8/− 1.1) ($p < 0.05$) (Table 2). There was no significant difference between POAG, PXG and control groups in terms of iVD ($p > 0.05$) (Table 2). There was also no difference between the POAG and PXG patient groups in terms of pVD values ($p > 0.05$); however, both POAG and PXG patients had significantly lower pVD values (mean, sup, inf, 4-sup, 4-temp, 4-inf, 4-nas) compared to the control group (median mean pVDs were: 50.0, 50.3, 53.1, respectively, CI: − 8.6/− 1.4) ($p < 0.05$) (Table 2).

In terms of parafoveal vessel density, while there was no significant difference between the POAG and other groups (PXG and control group) ($p > 0.05$), there was a significant decrease in the PXG group compared to the control group, except for the nasal quadrant values ($p < 0.05$) (Table 3).

No correlation was found between the rim area and the mean pVD in all three groups ($p > 0.05$). While there were significant positive correlations between RNFL thickness and pVD in all examinations in the PXG group ($p < 0.001$), in the POAG group, a strong positive correlation was detected in all quadrants except for the temporal quadrant ($p < 0.05$). There was no significant correlation between RNFL thickness and pVD values in the control group ($p > 0.05$) (Table 4). Correlation coefficients are shown in Table 4.

There was a significant positive correlation between pfVD and RNFL in the PXG group, but there was no correlation in the POAG group (Table 5). No correlation was found between disk area values and the mean pVD and tVD in the POAG, PXG and control groups ($p > 0.05$). While there was a moderate negative correlation between age and pVD only in the control group ($p = 0.002$), no correlations were observed between age and pVD in the glaucoma groups ($p > 0.05$). Lastly, we also found no significant correlations between the pVD and IOP values in any group ($p > 0.05$) (Table 6).

All patients in the glaucoma group were using IOP lowering drops. Beta-blockers were used by 32 (64.0%) patients, prostaglandin analogs by 26 (52.0%) patients, carbonic anhydrase inhibitors by 15 (30%) patients and alpha-agonists by 13 (26%) patients. Please note that more than one active substance were used in the majority of patients; hence the calculated rates have a higher total than 100%. There was no significant difference between the

Table 2 Comparison of total area, intrapapillary and peripapillary vessel density

Parameters	Groups			*p* value		
	POAG[1]	PXG[2]	Control[3]	p^{1-2}	p^{1-3}	p^{2-3}
tVD	45.4 (42.7–48.0)	45.9 (39.5–48.7)	50.0 (48.1–50.7)	1.000	**0.004**	**0.004**
iVD	45.2 (40.1–49.2)	42.0 (39.9–44.7)	45.7 (43.7–48.0)	0.391	1.000	0.160
pVD						
Mean	50.0 (42.6–52.4)	50.3 (42.9–52.9)	53.1 (51.8–54.2)	0.898	**0.010**	**0.014**
Sup	50.9 (40.9–52.7)	49.9 (42.9–53.9)	53.6 (51.1–54.6)	0.856	**0.007**	**0.011**
Inf	49.2 (44.4–53.4)	51.3 (43.2–52.9)	53.0 (52.6–53.7)	0.698	**0.022**	**0.032**
4-Sup	50.0 (40.0–56.0)	51.0 (42.0–54.0)	53.0 (52.0–54.0)	0.877	**0.014**	**0.030**
4-Temp	48.0 (40.0–53.0)	50.0 (44.0–53.0)	54.0 (50.0–56.0)	0.785	**0.002**	**0.025**
4-Inf	53.0 (48.0–56.0)	50.0 (48.0–56.0)	55.0 (54.0–56.0)	0.474	**0.044**	**0.018**
4-Nas	49.0 (43.0–53.0)	50.0 (41.0–52.0)	53.0 (47.0–55.0)	0.675	**0.035**	**0.042**

Statements in bold indicate statistically significant results

Values are shown as median (25–75%)

tVD total area vessel density, *iVD* ıntrapapillary vessel density, *pVD* peripapillary vessel density, *Mean* average, *Sup* superior half, *Inf* ınferior half, *4-Sup* 4 quadrant superior, *4-Inf* 4 quadrant ınferior, *4-Temp* 4 quadrant temporal, *4-Nas* 4 quadrant nasal

Table 3 Comparison of parafoveal vessel density and central macular thickness

Parameters	Groups			*p* value		
	POAG[1]	PXG[2]	Control[3]	p^{1-2}	p^{1-3}	p^{2-3}
Parafoveal VD						
Sup	44.1 ± 6.6	42.8 ± 5.6	47.1 ± 4.8	1.000	0.132	**0.027**
Temp	43.7 ± 6.0	42.5 ± 5.0	47.1 ± 5.3	1.000	0.077	**0.011**
Inf	44.5 ± 7.0	42.3 ± 5.6	47.3 ± 5.1	0.894	0.222	**0.042**
Nas	42.1 ± 5.8	41.8 ± 4.8	44.2 ± 4.7	1.000	0.376	0.294
Mean	43.6 ± 5.7	42.6 ± 4.7	46.4 ± 4.5	1.000	0.101	**0.024**
CMT	254.7 ± 16.1	249.7 ± 17.5	259.1 ± 16.0	0.728	0.295	0.094

Statements in bold indicate statistically significant results

Values are shown as mean ± standard deviation

VD vessel density; *CMT* central macular thickness

Table 4 Correlations between retinal nerve fiber layer thickness and peripapillary vessel density

Parameters	POAG*		PXG*		Control‡	
	r	*p*	*r*	*p*	*r*	*p*
Rim area/pVD mean	0.350	0.093	0.363	0.075	0.165	0.441
RNFL mean/pVD mean	0.878	**< 0.001**	0.883	**< 0.001**	0.357	0.081
RNFL sup/pDD sup	0.837	**< 0.001**	0.862	**< 0.001**	0.295	0.161
RNFL inf/pDD inf	0.894	**< 0.001**	0.803	**< 0.001**	0.352	0.092
RNFL 4-sup/pVD 4-sup	0.807	**< 0.001**	0.703	**< 0.001**	0.169	0.430
RNFL 4-inf/pVD 4-inf	0.822	**< 0.001**	0.743	**< 0.001**	0.282	0.182
RNFL 4-temp/pVD 4-temp	0.311	0.130	0.799	**< 0.001**	0.094	0.661
RNFL 4-nas/pVD 4-nas	0.492	**0.015**	0.703	**< 0.001**	0.208	0.330

Statements in bold indicate statistically significant results

*With Spearman correlation analysis

‡Calculated free from age effect with partial correlation analysis

Table 5 Correlations between retinal nerve fiber layer thickness and parafoveal vessel density

Groups	Parafoveal VD–RNFL	
	r	*p*
POAG	0.123	0.558
PXG	0.823	**< 0.001**
Control	0.076	0.717

Statements in bold indicate statistically significant results

r = correlation coefficient, *p* = statistical significance

Table 6 Correlations between disk area, age, IOP and vascular density in patient groups and control group

Parameters	POAG pVD (mean)	PXG pVD (mean)	Control pVD (mean)
Disk area			
r	0.275	0.002	0.107
p	0.183	0.994	0.618
Age			
r	− 0.041	− 0.041	− 0.598
p	0.846	0.846	**0.002**
IOP			
r	0.053	0.310	-0.307
p	0.800	0.140	0.135

Statements in bold indicate statistically significant results

IOP intraocular pressure, *pVD* peripapillary vessel density

r = correlation coefficient, *p* = statistical significance

POAG and PXG patient groups in terms of therapies (Table 7).

Comparisons were made between subgroups created according to the anti-glaucomatous medications used by the patients (Table 8). The results of iVD and mean pf-VD were found to be significantly lower in the patient group using carbonic anhydrase inhibitors ($p < 0.05$). tVD and mean pVD were significantly lower in patients using beta-blockers ($p < 0.05$). Significantly higher pf-VD values were found in patients using the prostaglandin analogs compared to those who did not ($p < 0.001$). No significant difference was found in any of the measurements in patients who were using alpha-agonists ($p > 0.05$) (Table 8).

Table 7 Comparison of antiglaucomatous medications used in POAG and PXG groups

Drugs used	POAG (n = 25) n (%)	PXG (n = 25) n (%)	p value*
Beta-blockers	16 (64.0)	16 (64.0)	1.000
Prostaglandin analogs	15 (60.0)	11 (44.0)	0.258
Alpha-agonists	8 (32.0)	5 (20.0)	0.333
Carbonic anhydrase Inhibitors	5 (20.0)	10 (40.0)	0.123

*comparison made with the Chi-Square test

Table 8 The comparison of vascular density values with regard to type of antiglaucamatous medication

Medications	tVD	iVD	pVD mean	pf-VD mean
CAI				
Present (n = 32)	42.1 ± 6.8	40.3 ± 4.2	45.0 ± 8.0	40.5 ± 4.5
Absent (n = 18)	45.4 ± 5.5	45.3 ± 5.0	48.6 ± 7.1	44.2 ± 5.1
p value	0.115	**0.002**	0.156	**0.024**
Beta-blocker				
Present (n = 32)	42.9 ± 6.3	43.1 ± 5.2	46.0 ± 7.8	50.3 ± 6.1
Absent (n = 18)	47.1 ± 4.7	45.1 ± 5.3	50.3 ± 6.1	43.7 ± 5.3
p value	**0.013**	0.221	**0.041**	0.537
Prostaglandin analogs				
Present (n = 26)	45.4 ± 6.0	44.8 ± 5.0	48.4 ± 7.8	45.5 ± 5.0
Absent (n = 24)	43.4 ± 6.1	42.7 ± 5.4	46.5 ± 7.2	41.2 ± 5.1
p value	0.113	0.113	0.190	**< 0.001**
Alpha-agonist				
Present (n = 13)	44.5 ± 5.3	46.8 ± 5.5	47.1 ± 6.7	42.3 ± 4.3
Absent (n = 37)	44.4 ± 6.4	42.7 ± 4.8	47.6 ± 7.8	43.4 ± 5.5
p value	0.388	0.053	0.426	0.370

Statements in bold indicate statistically significant results

Values are given as mean ± standard deviation

CAI carbonic anhydrase inhibitor, *VD* vascular density, *t* total area, *i* intrapapillary, *p* peripapillary, *pf* parafoveal

Discussion

The OCTA modality, a new functional extension of optical coherence tomography, is a noninvasive imaging method that does not require contrast agent injection and has the ability to visualize and quantitatively evaluate vascularity in the retina, ONH and peripapillary region with motion contrast created by erythrocytes [7].

The presence of DM and HT for all groups was determined as the exclusion criteria in our study. OCTA examinations in DM patients without diabetic retinopathy (DRP) reveal that there may be early vascular changes in DM patients without DRP findings [8]. We aimed to eliminate the confounding effects of these diseases on the vasculature of patients by excluding them from the study.

The mean age of the PXG group was significantly higher than the other two groups ($p < 0.001$). This is an inevitable result due to the fact that PXG is seen in older ages [1, 9]. The statistical analyses were carried out by adjusting for the age effect because of possible changes in vascular density with aging.

We could not find any significant difference between the glaucoma groups in terms of the type of antiglaucomatous medications they use. It is mentioned that the use of antiglaucomatous drops is effective on VD parameters [10]. Although this effect is likely, the similarity in drug use among our glaucoma groups suggested that the effects of drugs on parameters in patient groups may be rather subtle.

No significant difference was found between the groups in terms of IOP values in our study. The effect of IOP alterations on VD has not been clarified yet. Hollo et al. reported in a case series of high-pressure glaucoma patients that, as a result of lowering IOP, peripapillary VD increased compared to pretreatment values [11]. For this reason, we believe that the lack of significant difference between the groups in terms of IOP eliminates the possibility of compression and

decreased perfusion that may occur in the vessels due to very high IOP.

Although the POAG and PXG groups did not reach statistically significant levels of difference in terms of rim area, cup/disk ratio and RNFL thickness, the values of RA and RNFL thickness were lower, and cup/disk ratios were higher in the POAG group. This suggests that the severity of glaucoma may have been more advanced among patients in the POAG group.

We examined the mean, superior–inferior half-area and four quadrant values for pVD between POAG, PXG and control groups separately, and found that there were significant reductions in all areas examined in both glaucoma groups compared to the control group. Similarly, Lommatzsch et al. analyzed overall pVD and pVD values from 6 sectors (superonasal, superotemporal, nasal, inferonasal, inferotemporal, temporal) in glaucoma patients. They found a significant decrease in overall average value and also sector-based values [12]. Mansoori and colleagues compared early POAG patients and healthy individuals and they found significantly lower mean pVD in glaucomatous eyes compared to controls. However, they found a significant decrease only in superotemporal and inferotemporal sectors. This may be due to the fact that Mansoori et al. included early glaucomatous cases in their studies [13].

There are studies comparing glaucoma patients with healthy controls, as well as studies that include patients with a suspicion for glaucoma and also patients with pseudoexfoliation syndrome (PXS) in the literature. Triola et al. found significant decrease in mean and quadrant values (excluding nasal quadrant) in pVD measurements between POAG, glaucoma suspect and healthy control groups [14]. Suwan et al. evaluated VD values between PXS, PXG, POAG and control groups and detected a gradual decrease in peripapillary capillary VD values from control group to PXS, POAG and PXG groups [15]. Suwan et al. predicted that PXG patients had a lower pVD value than POAG patients because of increased vascular resistance caused by the pseudoexfoliative material in PXG [15]. This also supports previous studies that reported impaired ocular blood flow and increased vascular resistance by non-OCTA methods [16].

We found that tVD values were significantly lower in the POAG and PXG groups compared to controls. In addition, if our results are examined in terms of pVD and tVD values, it can be seen that tVD values show higher statistical significance in group comparison compared to pVD values. For instance, Yarmohammadive and colleagues measured the diagnostic capability of VD parameters in healthy, suspicious glaucoma and glaucoma eyes, and stated that tVD had better diagnostic accuracy compared to pVD in differentiating glaucoma patients from healthy patients [17].

We found a correlation between overall, half-area and all quadrant pVD values and same area RNFL thickness in both glaucoma groups. There was also a strong positive correlation between pVD values and same area RNFL thickness in both glaucoma groups (except for temporal quadrant for POAG). Our results are compatible with the correlation analysis evaluations in the literature [13, 14, 18].

Another parameter examined in our study is parafoveal VD. Despite the relatively high RNFL thickness values in the PXG group compared to the POAG group, the greater loss in parafoveal VD suggests that PXG affects vascular structures in the parafoveal area more than POAG. This situation can be associated with mechanisms that cause an increase in vascular resistance in PXG [19]. The significant positive correlation we found between pfVD and RNFL among patients with PXG seems to support these findings. Although pVD values were decreased significantly in the POAG group, there was no significant decrease in pfVD; suggesting that the disruption of vascular autoregulation mechanisms in the POAG group resulted in a lesser effect on parafoveal area, when compared to the effect of increased vascular resistance in PXG. There is a need for longitudinally designed studies to elucidate this matter.

In their study of perimetric glaucoma patients and healthy individuals, Takasuwa et al. [10] examined the superficial capillary plexus (SCP) and deep capillary plexus (DCP) vascularity in the macular area. As a result of this study, they found that SCP VD values were significantly lower in the glaucoma group than in controls and they stated that glaucoma affects the superficial plexuses rather than the deep plexuses [10]. Only superficial parafoveal VD was evaluated in our study. The reason that we focused on this area was the fact that this area feeds the retinal ganglion cells [20]; thus, alterations can be mostly expected in this region. Furthermore, we examined the parafoveal area because the ganglion cell complex in the parafoveal

area is thicker and due to the fact that SCP has an intersecting structure on the parafoveal ring [20].

We found a significant negative correlation between age and mean pVD only in the healthy control group and not in glaucoma groups. In the literature, there are similar results to our study in terms of the correlation between age and pVD values in patients with glaucoma [18, 21]. However, there are also studies that have detected a negative correlation between these values in patients with glaucoma [12].

When evaluating the results, possible parameters that may affect OCTA measurement values should not be ignored. VD parameters are more likely to be affected by some factors associated with the patient or scanning protocol than structural parameters [22].

Cataract surgery is among the factors that may affect VD. In a study by Pilotto et al. [23], where the effects of inflammation after non-complicated cataract surgery on OCTA perfusion was assessed, it was found that all OCTA parameters returned to normal at post-op 90 days. In our study, the PXG group had a higher frequency of patients with pseudophakia; however, we only included patients that had undergone surgery at least 6 months prior to study inclusion in order to eliminate the possible effects of inflammation.

To provide dilation before OCTA measurement, we only used a 0.5% solution of tropicamide and phenylephrine was avoided. Phenylephrine is a selective α1-adrenergic receptor agonist with vasoconstrictive effect. In their study investigating the effect of the use of tropicamide and phenylephrine on retinal perfusion, Cheng et al. did not detect any change in VD with the use of tropicamide. But, they detected a statistically significant decrease in peripapillary VD with the use of phenylephrine [24]. In the literature, there are several studies reporting that neither phenylephrine nor tropicamide cause changes in VD [25, 26]; however, in order to reduce the possibility of such effects, we utilized the safer option, tropicamide. The use of antiglaucomatous drops is another reason that may affect VD measurements. Due to ethical reasons, none of the treatments were altered or stopped during the course of this study. Therefore, it was not possible to evaluate our results independently of antiglaucomatous medication use. However, we think that this effect was minimal in our study, since no differences were found between the POAG and PXG groups when parameters were compared with regard to drug types.

We performed subgroup analyzes to compare the possible effects of the antiglaucomatous agents used. However, the limited number of patients in subgroups and the fact that some patients were using more than one agent are important limitations of this analysis. However, there are very few studies reporting such findings; thus, we have presented our results to at least provide some idea about these agents and their effects. We found a significant decrease in tVD and overall pVD values in those using beta-blockers. Similarly, Takusagawa et al. [10] found a significant relationship between beta-blocker eye drops and reduced macular superficial VD in glaucomatous eyes. However, it would not be correct to assume that beta-blockers cause a direct decrease in vascularity and VD based on these results alone. It is possible to use beta-lockers with more frequently in cases where glaucomatous changes are more severe and this may have affected our VD results.

We found a statistically significant increase in all quadrant values of parafoveal VD in patients using prostaglandin analogs. The vasodilator effects of prostaglandin analogs can cause this result [27]. In previous studies examining this issue with regard to ONH perfusion, the use of prostaglandin analogs led to an increase in ocular perfusion pressure, and this characteristic has been predicted as an advantage to prevent glaucomatous damage [28, 29]. No OCTA study investigating this subject with asimilar approach to ours was found in the literature. There is a need for clinical studies using OCTA.

We did not come upon any research concerning the use of carbonic anhydrase inhibitors and OCTA imaging. However, Dong and colleagues observed increased ocular blood flow with brinzolamide via measurements performed with a laser speckle flowmeter. The authors associated this finding with the direct vasodilation effect of brinzolamide through the suppression of intracellular Ca + 2 release [30]. The low pfVD and iVD values among CAI users in our study were not in agreement with previous non-OCTA studies. However, we believe that direct conclusions concerning VD parameters should not be drawn from results associated with ocular blood flow. Therefore, we believe there is a need for further studies employing OCTA-based assessments in a higher number of individuals.

Our study has some limitations. A relatively limited sample size is one of them; however, as discussed

earlier, this sample was obtained with rigid inclusion/exclusion criteria and the study population can be viewed as an accurate representation of patient populations without eye-related or systemic comorbidities. Another limitation is that we did not include the axial lengths of patients as a study parameter. Although axial length differences may affect structural and vascular parameters [31], we tried to minimize this limitation by limiting refraction interval values in the inclusion criteria. Despite our caution, it is important to note that the literature is conflicted on this topic. For instance, Lommatzsch et al. did not find a significant relationship between refractive error and tVD and pVD values [12]; whereas Sakaguchi et al. found a significant negative correlation between axial length and pVD [18]. In addition, the lack of blood pressure measurements in the current study is another limitation. Since none of our patients reported a history of hypertension, we believe that the 15-min resting duration before OCTA measurements were sufficient to nullify possible efort-related alterations in blood pressure. It is possible that there is a relationship between ONH blood flow and blood pressure; however, studies that have not determined a relationship between ONH VD values and blood pressure also exist [32].

Both of the glaucoma groups assessed in our study (POAG and PXG) demonstrated decreased tVD, tVD (mean and quadrants) compared to controls. We also determined significant correlations between RNFL and both the mean and quadrant values of pVD. These results show the close relationships between structural and vascular parameters.

The decreased VD values observed in the parafoveal measurements of patients with PXG suggests that the pathophysiology of PXG is associated with the increased vascular resistance caused by the effects of pseudoexfoliative material.

Despite the relatively limited number of patients in our study, we were able to assess the relationships between type of medication and VD values. To our knowledge, there are very little data on this topic and we believe that the OCTA VD results in this study could prove important for further research. With this analysis, we found that beta-blocker recipients had significantly lower tVD and mean pVD values compared to those not using beta-blockers ($p < 0.05$). Furthermore, we also found that patients using prostaglandin analogs had higher parafoveal VD ($p < 0.001$).

In our study, we focused on SCP and RCP, which are responsible for retinal ganglion cell nutrition, and RNFL thickness to evaluate glaucomatous changes. The low VD we found in glaucomatous eyes and the correlation of these results with structural OCT parameters is compatible with the majority of the literature.

Despite current theories, glaucoma etiopathogenesis is still a controversial issue. There are opinions suggesting that there are vascular changes as a result of glaucoma-induced pathology and increased IOP as well as opinions arguing that blood flow changes are not a consequence of the disease but part of the cause [33]. Similar differences of opinion exist in VD studies. In addition to studies suggesting that neurodegeneration occurs before vascular damage in glaucoma (and therefore vascular losses are secondary to the loss of RNFL), there are also studies indicating that vascular damage is a glaucomatous factor and an early marker that exists before structural damage [34]. Considering our study and literature data, it is understood that there is a relationship between structural parameters and vascular parameters. In this context, long-term studies with more cases are needed to determine whether low VD in glaucoma patients develop before optic nerve damage, and also to determine the role of VD parameters in glaucoma diagnosis and follow-up.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Ethics committee approval was uploaded in supplementary (E-18-1770—University of Health Sciences Turkey, Clinical Research Ethics Committee).

Informed consent Informed consent was obtained from all individual participants included in the study.

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