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Relationship Between Chorioretinal Vascular Density And The Visual Field In Glaucomatous Eyes

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Abstract
Aim: *the chorioretinal vascular density (crvd) in glaucomatous eyes was investigated using widefield swept-source optical coherence tomography angiography (ss-octa), and the differences between glaucomatous and normal eyes along with the relationship glaucomatous eyes with visual field defects were examined. This study aims to analyze the differences between glaucomatous and normal eyes.*
Methods: *forty normal eyes with no anterior eye abnormalities, no ocular media abnormalities, normal open angles, spherical power of −6.0–<+3.0 d, and best-corrected visual acuity of ≥20/25 were included in this cross-sectional study. Participants' ages ranged from 20 years to 73 years. Images obtained using widefield ss-octa of 96 glaucomatous and 40 normal eyes were divided into nine segments. The crvd of the superficial and deep retinas and choriocapillaris were calculated using imagej software. The differences between normal eyes and the relationship between the crvd and visual field of glaucomatous eyes were examined.*
Results: *crvd in the superficial and deep retinas of glaucomatous eyes was significantly lower ($p < 0.02$) than that in normal eyes in each of the nine segments ($p < 0.02$). There was no significant difference in crvd of the choriocapillaris between normal and glaucomatous eyes. A significant correlation was observed between visual field defects in the superficial and deep layers but not in the choriocapillaris layer.*
Conclusion: *in glaucomatous eyes, decreased crvds in the superficial and deep retinas and a correlation with visual field defects were observed; however, no decrease in crvd in the choriocapillaris layer and no correlation with visual field defects were observed.*
Keywords: *chorioretinal vascular density, glaucoma, swept-source optical coherence tomography angiography, imagej software, choriocapillaris*

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I. Introduction

Glaucoma is a multifactorial disease that develops not only due to high intraocular pressure, but also due to various risk factors, such as optic nerve fragility, genetic abnormalities, neurodegeneration, immune abnormalities, and ocular circulation. Many studies, including a large-scale epidemiological study[1], have demonstrated that the ocular circulation is significantly associated with the prevalence, onset, and progression of glaucoma.

The optic disc is nourished by the central retinal artery and the short posterior ciliary artery. The central retinal artery mainly nourishes the optic nerve fiber layer, whereas the short posterior ciliary artery nourishes the lamina cribrosa region. Narrowing of the central retinal artery diameter is associated with new-onset glaucoma 10 years later (adjusted odds ratio: 1.76–1.87)[2, 3]. According to a meta-analysis investigating the factors associated with glaucoma progression, high resistance to ocular blood flow is associated with disease progression. In normal-tension glaucoma, optic disc hemorrhage, parapapillary atrophy, cerebral infarction, and hypertensive migraine are associated with the progression of visual field impairment[4]. Thus, intraocular pressure and ocular circulation are important risk factors for the progression of visual field impairment. Conventionally, fluorescent fundus angiography using contrast media such as fluorescein sodium and indocyanine green has been mainly used for investigating circulatory disorders of the fundus; however, there are also concerns regarding invasive injections or anaphylaxis after injection. Laser speckle flowgraphy[2, 5], the Doppler ultrasound method, the microsphere method, and the hydrogen clearance method can also be used for ocular blood flow measurement; however, optical coherence tomography angiography (OCTA) without contrast enhancement is able to measure the vascular density in each layer of the ocular fundus without physical invasion, providing high-quality and reproducible images within seconds, which can be converted to data[6].

Based on the relationship between vascular density and glaucoma demonstrated by OCTA, correlations between decreased peripapillary capillary and macular retinal vascular densities and changes in visual field impairment have been reported[7–9]. In the present study, the chorioretinal vascular density (CRVD) up to the periphery of the retina of glaucomatous eyes was measured using widefield OCTA and compared with that of normal eyes, and its correlation with visual field defects was examined.

II. Subjects And Methods

Subjects

Under the approval of the Ethical Review Board of Tokai Eye Clinic, 40 normal eyes of 20 normal participants and 96 eyes of 48 patients with glaucoma, aged 20–73 years, who gave their written informed consent from June 2000–December 2000 in compliance with the Declaration of Helsinki, were investigated. Per the Japan Glaucoma Society Guidelines for Glaucoma, 4th Edition[10], glaucomatous eyes, defined as primary open-angle glaucoma, were investigated (Table 1). The participants presented with morphological characteristics (enlargement of the optic disc cupping, thinning of the peripheral region, and retinal nerve fiber layer defects) in the optic nerve head and retinal nerve fiber layer, had no other diseases or congenital abnormalities, and were open-angle type without angle functional abnormalities. In addition, 96 eyes of 48 subjects with no anterior eye abnormalities, spherical power of −6.0 D–<+3.0 D, cylindrical power of ±2.5 D, best-corrected visual acuity of at least 40/50, and no history of intraocular surgery were investigated. Further, 40 normal eyes of 20 subjects with no anterior eye abnormalities, no ocular media abnormalities, with normal open angles, spherical power of –6.0 D–<+3.0 D, cylindrical power of ±2.5 D, best-corrected visual acuity of at least 20/25, and no history of intraocular surgery were investigated. Table 1 shows the background, age, sex, spherical equivalent, visual acuity, and intraocular pressure of the subjects.

Table 1 Under the approval of the Ethical Review Board of Tokai Eye Clinic, 40 normal eyes of 20 normal subjects and 96 eyes of 48 patients with glaucoma aged 20–73 years were included in this study

Characteristics of the participants		
Parameters	Normal	Glaucoma
	20 cases (40 eyes)	48 cases (96 eyes)
Age (y)		
Mean ± SD	51.5 ± 13.8	53.5 ± 12.1
Range	23–69	20–73
Gender, n (%)		
Female	14 (70%)	25 (52%)
Male	6 (30%)	23 (48%)
Spherical equivalent (D)		
Mean ± SD	−1.76 ± 1.9	−2.48 ± 2.29
Range	−5.75 to +1.25	−6.15 to +2.5
Best corrected visual acuity (log MAR)		
Mean ± SD	0.12 ± 0.07	0.14 ± 0.05
Range	−0.2 to +0.1	−0.1 to +0.2
IOP (mmHg)	14.5 ± 3.45	17.91 ± 5.11
Range	9–18	12–21
IOP: intraocular pressure.		

Methods

The CRVD in glaucomatous eyes, including the periphery of the fundus, was investigated using widefield swept-source OCT angiography (SS-OCTA; PLEX Elite 9000 Zeiss), and the differences between glaucomatous and normal eyes and the relationships with glaucomatous visual field defects were examined.

A 12 mm × 12 mm wide-angle SS-OCTA image of 96 glaucomatous eyes and 40 normal eyes was divided into nine segments, and the CRVD of the superficial retinal vascular layer, deep retinal vascular layer, and choriocapillaris was calculated using ImageJ software. The differences between glaucomatous and normal eyes and the relationships between the CRVDs and visual field defects (Humphrey Field Analyzer 30-2) (grayscale) of glaucomatous eyes were examined (Figures 1 and 2). Image analysis was performed by a single investigator. The *en face* image of each layer, which was divided into nine segments, was processed using ImageJ and binarized using Otsu's method[11] by the same investigator. Binarization was used to obtain the area

ratio of the white part, which corresponds to the blood vessel as the vascular density.

Figure 1 A: 12-mm × 12-mm wide-angle SS-OCTA image of 96 glaucomatous eyes and 40 normal eyes was divided into nine segments, and the CRVD of the superficial retinal vascular layer, deep retinal vascular layer, and choriocapillaris were calculated using ImageJ software.

Figure 2 Grayscale image and images of each layer of the retina. Vascular densities of the superficial, deep, and choriocapillaris layers were examined.

III. Results

Figure 3 shows a comparison between the CRVD of normal and glaucomatous eyes. Comparisons were performed using the Mann–Whitney U test, with the significance level set at 5%. The CRVDs of the superficial and deep retinal layers in glaucomatous eyes were significantly lower than those in normal eyes. There were no significant differences in the choriocapillaris layer between ocular fundus sections 2, 3, 4, 5, 6, and 9 (Table 2).

Table 2 The CRVDs of the superficial and deep retinal layers in glaucomatous eyes were significantly lower than that in normal eyes. There were no significant differences in the choriocapillaris layer between the ocular fundus sections 2, 3, 4, 5, 6, and 9

		Normal: 40 eyes		Glaucomatous: 96 eyes			
	Fundus	Median	Range	Median	Range	Statistics	*P*-value
Superficial	1	42.5	56.3	33.0	68.2	−3.921	0.000
	2	47.7	46.4	34.3	56.7	−4.671	0.000
	3	46.6	48.1	36.7	65.7	−4.962	0.000
	4	40.8	63.2	33.8	65.4	−3.759	0.000
	5	39.0	58.2	30.9	66.9	−3.405	0.001
	6	40.6	44.0	29.6	55.0	−5.020	0.000
	7	43.4	37.5	30.4	46.0	−5.640	0.000
	8	44.3	56.8	33.2	66.2	−4.504	0.000
	9	32.0	41.0	23.1	69.4	−3.592	0.000
Deep	1	48.7	42.7	38.0	87.3	−4.370	0.000
	2	45.9	45.9	37.6	87.2	−3.740	0.000
	3	47.0	42.0	37.7	90.6	−3.735	0.000
	4	45.9	53.6	37.9	77.2	−3.620	0.000
	5	42.7	53.0	33.2	83.1	−3.162	0.002
	6	40.2	36.2	32.8	60.3	−3.993	0.000
	7	44.8	33.2	33.7	63.0	−4.738	0.000
	8	48.2	46.4	35.6	82.2	−5.521	0.000
	9	23.0	36.6	14.9	96.9	−3.252	0.001
Choriocapillaris	1	37.4	43.1	32.6	60.3	−1.994	0.046
	2	35.4	40.7	31.7	51.6	−0.970	0.332
	3	35.5	34.0	33.2	44.2	−1.552	0.121
	4	35.5	45.7	33.6	40.0	−1.848	0.065
	5	36.5	31.3	89.7	96.1	−1.844	0.065
	6	35.3	24.4	32.7	42.8	−1.748	0.080
	7	38.7	34.5	35.3	45.4	−2.469	0.014
	8	41.3	42.3	33.8	53.4	−3.974	0.000
	9	28.9	42.5	25.9	49.1	−1.531	0.126

Figure 3 A comparison between the CRVD of normal and glaucomatous eyes performed using the Mann–Whitney U test, with the significance level set at 5%. The CRVDs of the superficial and deep retinas layers in glaucomatous eyes were significantly lower than those in normal eyes. There were no significant differences in the choriocapillaris layer between the ocular fundus sections 2, 3, 4, 5, 6, and 9.

Figures 4–12 show the relationships between the grayscale and CRVD of glaucomatous eyes in each of the nine segments and examine their relationships. In fundus section 1, positive correlations were observed in the superficial (correlation coefficient: 0.232), deep (0.235), and choriocapillaris (0.241) layers (Figure 4).

Figure 4 Figures 4–12 are the results of examining the relationship between the grayscale and CRVO of glaucomatous eyes into nine parts and examining their relationships. In fundus section 1, a significant correlation was observed in the superficial (correlation coefficient: 0.232), deep (0.235), and choriocapillaris (0.241) layers.

In fundus section 2, significant correlations were observed in the superficial (0.661) and deep (0.513) retinal layers. No significant correlation was observed for the choriocapillaris layer (0.191) (Figure 5). In fundus section 3, significant correlations were observed in the superficial (0.622) and deep (0.513) retinal layers, whereas no significant correlation was observed in the choriocapillaris layer (0.119) (Figure 6). In fundus section 4, no significant correlations were observed in the superficial (0.147) and deep (0.150) retinal layers, whereas a negative correlation was observed in the choriocapillaris layer (–0.051) (Figure 7). In fundus section 5, significant correlations were observed in the superficial (0.473) and deep (0.453) retinal layers, whereas no significant correlation was observed in the choriocapillaris layer (0.106) (Figure 8). In fundus section 6, significant correlations were observed in the superficial (0.504) and deep (0.466) retinal layers and in the choriocapillaris layer (0.238) (Figure 9). In fundus section 7, significant correlations were observed in the superficial (0.508) and deep (0.418) retinal layers, whereas no significant correlation was observed in the choriocapillaris layer (0.106) (Figure 10). In fundus section 8, significant correlations were observed in the superficial (0.376) and deep (0.314) retinal layers and in the choriocapillaris layer (0.239) (Figure 11). In fundus section 9, significant correlations were observed in the superficial (0.241) and deep (0.192) retinal layers, whereas no significant correlation was observed in the choriocapillaris layer (0.137) (Figure 12).

Figure 5 In fundus section 2, a significant correlation was observed in the superficial (correlation coefficient: 0.661) and deep (0.501) layers. No significant correlation was observed in the choriocapillaris layer (0.191).

Figure 6 In fundus section 3, a significant correlation was observed in the superficial (0.622) and deep (0.513) layers, whereas no significant correlation was observed in the choriocapillaris layer (0.119).

Figure 7 In fundus section 4, no significant correlation was observed in the superficial (0.147) and deep (0.150) layers, whereas a negative correlation was observed in the choriocapillaris layer (-0.051).

Figure 8 In fundus section 5, a significant correlation was observed in the superficial (0.473) and deep (0.453) layers, whereas no significant correlation was observed in the choriocapillaris layer (0.106).

Figure 9 In fundus section 6, a significant correlation was observed in the superficial (0.504), deep (0.466), and choriocapillaris (0.238) retinal layers.

Figure 10 In fundus section 7, a significant correlation was observed in the superficial (0.508) and deep (0.418) retinal layers, whereas no significant correlation was observed in the choriocapillaris layer (0.106).

Figure 11 In fundus section 8, a significant correlation was observed in the superficial (0.376), deep (0.314), and choriocapillaris (0.239) retinal layers.

Figure 12 In fundus section 9, a significant correlation was observed in the superficial (0.241) and deep (0.192) retinal layers, whereas no correlation was observed in the choriocapillaris layer (0.137).

The CRVD was significantly lower in glaucomatous eyes than in normal eyes. This difference was significant in each of the nine sections. No significant differences were observed in the choriocapillaris layer. In the CRVD and grayscale (visual field), a significant correlation was observed in both superficial and deep layers of the fundus for sections 1, 2, 3, 5, 6, 7, 8, and 9, and a positive correlation was observed in fundus section 4. No significant positive correlation was observed in the choriocapillaris layer in fundus sections 2, 3, 4, 5, 7, and 9, whereas a significant negative correlation was observed in fundus section 4 (Tables 3 and 4).

Table 3 In CRVD (Chorioretinal Vascular Density) and grayscale (visual field)

	Superficialis		Deep		ORCC		Choriocapillaris	
Fundus	ρ1	*P*	ρ1	*P*	ρ1	*P*	ρ1	*P*
1	0.232	0.023	0.235	0.021	0.303	0.003	0.241	0.018
2	0.661	0.000	0.501	0.000	0.371	0.000	0.191	0.062
3	0.622	0.000	0.513	0.000	0.276	0.007	0.119	0.249
4	0.147	0.153	0.150	0.145	−0.048	0.641	−0.051	0.624
5	0.473	0.000	0.453	0.000	0.219	0.032	0.106	0.305
6	0.504	0.000	0.466	0.000	0.286	0.005	0.238	0.019
7	0.508	0.000	0.418	0.000	0.232	0.023	0.106	0.303
8	0.376	0.000	0.314	0.002	0.196	0.056	0.239	0.019
9	0.241	0.018	0.192	0.061	0.095	0.356	0.137	0.182
P : Significance level				96 glaucomatous eyes				
ρ: Spearman's rank correlation coefficient								

Table 4 Summary of Table 3. In the CRVD and grayscale, a significant correlation was observed in both superficial and deep layers in fundus sections 1, 2, 3, 5, 6, 7, 8, and 9. No significant positive correlation was observed in the choriocapillaris layer in fundus sections 2, 3, 4, 5, 7, and 9, whereas a negative correlation was observed in fundus section 4

Fundus	1	2	3	4	5	6	7	8	9
Superficial	+	+	+	-	+	+	+	+	+
Deep	+	+	+	−	+	+	+	+	+
Choriocapillaris	+	-	-	−	−	+	−	+	−
+:Significant difference −: No significant difference									

IV. Discussion

Hypotheses about the cause of glaucomatous optic nerve atrophy include the mechanical disorder theory[12] and the circulatory disorder theory[13]. There are two types of glaucoma: high-tension glaucoma and normal-tension glaucoma. High-tension glaucoma is a mechanical disorder of optic nerve fibers due to intraocular pressure, whereas normal-tension glaucoma involves intrapapillary circulation disturbances, lamina cribrosa fragility, oxidative stress, hypotension, and autoantibodies, while normative intraocular pressure is considered low. Preperimetric glaucoma with a strong circulatory disorder often progresses to glaucoma[5].

Splinter hemorrhage of the optic disc is considered an intrapapillary circulation disturbance. The Ocular Hypertension Treatment Study reported that hemorrhage is a sign of progression from ocular hypertension to primary open-angle glaucoma[14]. Pathologically, the first damaged site of the optic nerve in glaucomatous eyes is in the prelaminar area. It is hypothesized that disturbed blood flow in this area causes optic nerve atrophy[13].

OCTA can visualize minute retinal vessels via a decorrelation since it is sensitive to horizontal blood flow. Reproducibility is reported to be 4.3% for flow and 2.7% for density[15]. The correlation between blood flow and decorrelation is sigmoid[16]. When the interscan time is shortened, the region where the correlation between decorrelation and blood flow is established becomes wider, but the signals in the region with low blood flow are lost, leading to deterioration of the image quality. The blood flow is rapid in large blood vessels and saturated with the Plex-Elite Zeiss with an interscan time of 3.7 ms, which is suitable for quantitative analyses at the capillary level. However, very slow blood flow, such as vascular leakage, can be reversed by setting a threshold[17]. In the present study, these characteristics of OCTA were used, and CRVD was calculated using images up to the periphery of the fundus using ImageJ.

Clinical Study of Glaucoma and OCTA

Microsphere and laser speckle methods demonstrated that autoregulation of optic disc blood vessels is disrupted by glaucoma, leading to decreased blood flow and vascular density of the entire optic disc[18]. This has also been proven using OCTA[19, 20]. With OCTA, vascular analyses of layered tissues using *en face* images revealed that RPC falls off early[21] in line with a retinal nerve fiber layer defect[9], accompanied by decreased blood flow[22]. Furthermore, it has been reported that circulatory disorders occur in the superficial layer in the early stage of glaucoma and that the deep layer is protected by autoregulation[17].

Many studies using OCTA have reported decreased vascular densities in the optic disc;[7–9, 22–25] however, there are few reports on the detailed examination of the peripheral region of the retina. OCTA can

image the superficial, deep, and outer retinal layers and the choroid. The intraretinal layer is the most commonly affected region in glaucomatous eyes. Reading OCTA images in the order of the retinal superficial layer, deep layer, and choroid helps understand vascular disorders. A correlation between the foveal avascular zone and central visual field defects has been reported in glaucomatous eyes[25]. Enlargement of the foveal avascular zone and decreased vascular densities are also indicated at the site of visual field defects[26]. In glaucomatous eyes, changes occur mainly along the nerve fiber layer. The current OCTA is not equipped with vascular density measurement software that evaluates changes due to glaucoma. ImageJ was used to investigate the vascular density up to the periphery of the retina and the relationships between density and visual field defects. This study revealed that retinal circulation disorder, which is consistent with the site of visual field defects, may be involved in glaucomatous eyes. In the future, we would like to accumulate more data and pursue the reproducibility of blood vessel density quantification.

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