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Analysis of structural and vascular changes of the optic disc and macula in different stages of primary open angle glaucoma

Analiza strukturnih i vaskularnih promena optičkog diska i makule u različitim stadijumima primarnog glaukoma otvorenog ugla

Maja Petrović*, Mirko Resan[†‡], Gordana Stanković Babić[§], Tatjana Šarenac Vulović[‖], Marija Radenković*, Aleksandar Veselinović*, Marija Trenkić[§], Marija Cvetanović*

***Clinical Center Niš, Eye Clinic, Niš, Serbia ; [†]Military Medical Academy, Eye Clinic, Belgrade, Serbia; [‡]University of Defence, Faculty of Medicine of the Military Medical Acadamy, Belgrade, Serbia; [§]University of Niš, Faculty of Medicine, Niš, Serbia; [‖]University of Kragujevac, Faculty of Medicine, Kragujevac, Serbia**

Abstract

Background/Aim. It is possible that patients with open-angle glaucoma be asymptomatic in the early stage of the disease. The aim of this study was to determine the structural and vascular changes of the optic disc (OD) and macula in healthy and primary open-angle glaucoma (POAG) eyes, detected by optical coherence tomography (OCT) and optical coherence tomography angiography (OCTA) as well as the correlation of the OCT and OCTA measurements and their association with the presence of POAG. **Methods.** A total of 196 eyes were included and classified into four groups, out of them 48 were healthy eyes, 51 eyes were with mild POAG, 50 eyes with moderate POAG, and 47 eyes with advanced glaucoma. All subjects underwent standard ophthalmic examination. OCT measured the mean, superior and inferior retinal nerve fiber layer (RNFL) thickness and macular ganglion cell complex (GCC). OCTA evaluated the vessel capillary density (VCD) in OD, foveal avascular zone (FAZ) and macular vessel density (VD) in the superficial (SL) and deep (DL) retinal vascular plexus. **Results.** Patient characteristics were similar except for decreased visual acuity, thinner corneas, higher intraocular pressure and higher cup/disc ratio in POAG patients. OCT results showed that RNFL and GCC thickness gradually decreased according to POAG severity. Within the assessment conducted by OCTA, VCD's value in OD also diminished with the progression of POAG, having the lowest value in patients with advanced glaucoma. The same pattern was observed in vessel density around FAZ and VD values. Comparing the structural and vascular changes, a significant positive correlation was found between RNFL thickness and VCD inside OD, and GCC and VD SL in the macular zone. **Conclusion.** OCT and OCTA allow of a noninvasive quantification of the structural and vascular changes in OD and the macular zone and accurately distinguish between healthy eyes and eyes with POAG, showing an association with the presence and progression of glaucoma.

Key words:
angiography; disease progression; glaucoma, open-angle; macula lutea; optic disc; tomography, optical coherence.

Apstrakt

Uvod/Cilj. U ranoj fazi bolesti, bolesnici sa glaukomom otvorenog ugla ne moraju imati simptome. Cilj rada je bio da se utvrde strukturne i vaskularne promene optičkog diska (OD) i makule u zdravim očima i očima kod bolesnika sa primarnim glaukomom otvorenog ugla (PGOU), koje su registrovane optičkom koherentnom tomografijom (OKT) i optičkom koherentnom tomografskom angiografijom (OKTA), kao i korelacija između OKT i OKTA parametara i njihova povezanost sa prisustvom PGOU. **Metode.** Ukupno je analizirano 196 očiju podeljenih u četiri grupe: 48 zdravih očiju, 51 sa blagim glaukomom, 50 sa umerenim glaukomom i 47 očiju sa uznapredovalim glaukomom. Svi ispitanici pregledani su standardnim oftalmološkim pregledom. Pomoću OKT merena je debljina prosečnog, gornjeg i donjeg sloja nervnih vlakana mrežnjače (SNVM) i ganglijskog ćelijskog kompleksa (GĆK) makule, a pomoću OKTA analizirala je gustina kapilarnih krvnih sudova (GKS) u OD, fovealna avaskularna zona (FAZ) i gustina krvnih sudova (GS) makule u površinskoj (P) i dubokoj (D) retinalnoj vaskularnoj mreži. **Rezultati.** Bolesnici sa PGOU imali su smanjenu oštrinu vida i smanjenu debljinu rožnjače, povišeni očni pritisak (OP) kao i povećani *cup/disc* (C/D) odnos. Rezultati OKT pokazali su da je prosečna debljina SNVM, kao i debljina GĆK smanjena kod bolesnika sa PGOU, posebno u kasnoj fazi bolesti. Ana

Correspondence to: Maja Petrović, Clinical Center Niš, Eye Clinic, Bulevar dr Zorana Đinđića 48, 18 000 Niš, Serbia.
E-mail: drpetrovicmaja@gmail.com

lizom OKTA utvrđeno je da se vrednosti GKS u OD takođe smanjuju sa napredovanjem PGOU, pokazujući najnižu vrednost kod bolesnika sa uznapredovalim glaukomom. Isto je zapaženo putem OKTA pregleda makule i praćenjem gustine krvnih sudova oko FAZ i vrednosti GS makule. Upoređivanjem strukturnih i vaskularnih promena, utvrđena je značajna pozitivna korelacija između debljine SNVM i GKS unutar OD, kao i GKS i GS makule u P retinalnoj vaskularnoj mreži. **Zaključak.** Primena OKT i OKTA omogućuje neinvazivnu kvantifikaciju strukturnih i vaskularnih promena u OD i makularnom predelu i precizno razlikovanje zdravih očiju od očiju sa PGOU, pokazujući povezanost sa prisustvom i progresijom glaukoma.

Ključne reči:
Angiografija; bolest, progresija; glaukom otvorenog ugla; žuta mrlja; optički disk; tomografija, optička, koherentna.

Introduction

Glaucoma represents the second leading cause of blindness worldwide [1]. It is a progressive optic neuropathy leading to preventable but irreversible visual defects [2]. It remains unclear whether the decreased retinal blood flow is the cause or result of an optic nerve glaucomatous damage [3]. However, there is growing evidence that vascular hypothesis, supported by reduced retinal blood flow and vessel diameter seen in glaucoma patients, can depict the pathogenesis of glaucoma [4, 5].

Recently, scholars have suggested that the vascular dysfunction in the optic disc (OD), also termed as optic nerve head, may have a crucial role in the development and progression of the open angle glaucoma (OAG) [6]. Considering the possibility for patients with glaucoma to be asymptomatic in the early stages, there is a serious risk for them to remain undiagnosed until the symptoms occur [4]. We hypothesize that it is important to visualize retinal microcirculation in order to confirm the diagnose and treat patients accordingly. The optical coherence tomography angiography (OCTA) was introduced as a reliable, non-invasive, rapid diagnostic procedure providing assessment of perfusion separately in various retinal layers, as well as in the OD [7]. Recent studies showed a reduced papillary vessel density (VD) in glaucomatous eyes [8] and suggested a possible correlation between the diameter of the retinal arterioles and the optic nerve damage [5]. Additionally, measuring the macular perfusion has the potential for detecting a reduced metabolic rate in dysfunctional retinal ganglion cells before they undergo apoptosis and cause the ganglion cell complex (GCC) to become thinner [9].

Structural changes in glaucoma patients can be detected using optical coherence tomography (OCT) as one of the noninvasive imaging technologies. OCT is designed to assess morphology and thickness of retinal layers, such as the innermost layers of the retina, which include the retinal nerve fiber layer (RNFL), ganglion cell layer, and inner plexiform layer. Diagnostic accuracy for glaucoma can be improved when macular OCT measurements focus particularly on the GCC [9], since it provides detailed information of retinal structure [10]. Both OCT and OCTA differentiate between healthy and glaucomatous eyes [11]. Nerve fiber layer and vessel diameter changes are pathophysiological manifestations of OAG without a strict cause-effect relationship in terms of which one appears first [4]. However, some studies confirmed their correlation showing OD blood flow decrease and RNFL thickness reduction are directly correlated in glaucoma patients [3]. Similar findings were observed within healthy subjects, showing retinal VD directly correlating with the RNFL thicknesses [10]. Furthermore, vessel density within the RNFL was lower in patients suffering from OAG [12].

The aim of this study was to evaluate structural and vascular changes of the OD and macula in healthy and OAG eyes, detected by OCT and OCTA, and to examine correlation of OCT and OCTA measurements and their association with the presence and stage of OAG.

Methods

This investigation was designed as a cross-sectional study. We examined a total of 196 eyes from 196 adult primary OAG (POAG) or POAG suspected patients examined at the Eye Clinic, Clinical Center Niš, Serbia from June 2019 to February 2020. The study was conducted according to the Declaration of Helsinki and the protocol was approved by the local Ethics Committee of the Clinical Center Niš.

Participants were enrolled after obtaining the informed consent and were divided into four groups. Group 1 consisted of 48 healthy eyes; group 2 had 51 eyes with stage 1 POAG; group 3 had 50 eyes with stage 2 POAG; and group 4 had 47 eyes with advanced glaucoma. Staging of POAG was performed by Hodapp-Parrish classification of severity of visual field defect. All subjects underwent standard ophthalmologic examination including visual acuity (VA) assessment, intraocular pressure (IOP) measurement using Goldmann applanation tonometry and a standard anterior segment slit lamp examination. Central Corneal Thickness (CCT) was measured by Pachymetry on Optical biometer and topography-keratometer OA-2000 Tomey.

All OCT and OCTA examinations were performed at Optovue apparatus, AngioVue Comprehensive Imaging system, using two patented technologies: Split Spectrum Amplitude Decorrelation Angiography (SSADA protocol) and Motion Correction Technology for reduction of artifacts by using software volume-based projection artifact removal (3D PAR). The vessel density measurements were determined with PAR correction only. The SSADA method is used to compare the consecutive B scans at the same location to capture the dynamic motion of the red blood cells using motion contrast. A trained examiner reviewed all the OCTA scans and only the images with good clarity, a signal strength index (SSI) of more than 50, with no residual motion were included for the analysis. Image cropping or local weak signal resulting from vitreous opacity or segmentation errors that could not be corrected were rejected.

The OCT measurement parameters in OD were RNFL defined as the thickness in micrometers and Cup/Disc area

(C/D) in mm^2. RNFL thickness was determined in OD mode in which data, along a 3.45-mm-diameter circle around the OD, was mapped using 12 concentric rings and 24 radial scans. Mean, superior, and inferior RNFL thicknesses were computed. The GCC scan covered a square grid of 6 × 6 mm on the central macula and was centered 1 mm temporal to the fovea. The GCC thickness was measured from the inner limiting membrane (ILM) to the posterior boundary of the inner plexiform layer. Mean, superior, and inferior GCC thicknesses were acquired.

During OCTA and retinal structure assessment, vessel capillary density (VCD) and vascular network density were evaluated in OD. Whole image (WI), inside disc (ID) and peripapillary density (PP) were acquired. In the foveal avascular zone (FAZ), OCTA analysed the following parameters: FAZ area, FAZ perimeter and foveal density (FD) – vessel density of the 300 μ width ring surrounding the FAZ, macular vessel density (VD) in the superficial (SL) and deep (DL) retinal vascular plexus with a 6 × 6 mm^2 macula scan were determined. Vessel density was calculated as the percent area occupied by flowing blood vessels in the selected region.

The retinal layers of each scan were segmented automatically by the AngioVue software to visualize the superficial retinal capillary plexuses in a slab from ILM to the inner plexiform layer (IPL) minus 10 μm. For this study, whole *en face* image VD (wiVD) was derived from the entire 6 × 6 mm^2 scan, and perifoveal VD was measured in an annular region centered on the fovea with an inner diameter of 1 mm and outer diameter of 3 mm.

The visual field was tested by the Standard Automatic Perimetry (SAP) 24-2 threshold test, at the Optopol PTS perimeter.

We included patients aged above 40 years with a confirmed diagnosis of glaucoma, without any chronic disease (diabetes, neurological disorders, cataracts, except the ones with the level of < 1 according to Lens Opacity Classification System) or medication use that could influence OCT and OCTA results. Other exclusion criteria were subjects with false positive and negative errors > 15% and fixation loss > 33%, on SAP, low signal intensity on OCT and poorly centered scan, anomalies in the anterior segment of the eye, trauma, chronic inflammation, retinal diseases, previously surgical and/or laser eye surgery. Pregnant women and women in the period of the lactation were also not considered.

Furthermore, eyes with a history of intraocular surgery (except uncomplicated cataract surgery or uncomplicated glaucoma surgery), coexisting retinal pathologic features, nonglaucomatous optic neuropathy, uveitis, or ocular trauma were also excluded from the study, as were individuals with a diagnosis of Parkinson's disease, Alzheimer's disease, or dementia and a history of stroke or diabetic or hypertensive retinopathy.

Data analysis and sample size determination

A total sample size was calculated *a priori* based on the G*Power software. A predicted effect size of 0.7 was smaller compared to the literature data where the difference in RNFL thickness between healthy subjects and glaucoma patients was 1.6482, the statistical power of 90% and significance level of 5%. We used a one-tailed *t*-test. Under these circumstances, a minimum of 82 patients (41 per group) were needed for this study.

In order to analyze the collected data, we used the standard methods of descriptive statistics. The differences between the mean values of continuous variables with normal distribution were assessed by a parametric ANOVA test. We used a nonparametric Kruskal Wallis H test for variables whose values did not follow a normal distribution. *Post hoc* analysis was done by a parametric Student's *t*-test and for variables whose values did not follow a normal distribution we used a non-parametric Mann-Whitney *U* test. To determine the differences in the incidence of certain categories, we used a χ^2 test or Fisher's test of the real likelihood for low frequencies. The presence of a relation between the variables of interest was analyzed by standard correlation analysis using the Pearson's correlation coefficient to determine the direction and strength of the connection/correlation. Binary logistic regression models were used to evaluate the association between OCT and OCTA parameters with the presence of POAG. In all analyses, *p*-value of less than 0.05 was considered as statistically significant.

Results

There were 76 (38.8%) males and 120 (61.2%) females, aged 66.4 ± 8.2 years (46–89 years), without a significant difference in average age between genders (66.7 ± 8.5 years male and 66.4 ± 8.0 years female).

Analysis was done between groups of glaucoma patients [148 (76.5%)] and healthy subjects [48 (24.5%)] with similar gender proportion and average age in both groups. The patients with glaucoma were divided according to glaucoma stage. There was a similar average age between the groups. The IOP was the highest and CCT the lowest in the third stage of glaucomatous eyes. Clinical characteristics of the examined groups of patients are shown in Table 1.

Table 1

Baseline characteristics of patients

Characteristics	Healthy subjects	Patients with glaucoma		
		first stage	second stage	third stage
Patients, n (%)	48 (24.5)	51 (26.0)	50 (25.5)	47 (24.0)
Male/female, n/n	13/35	17/33	23/27	23/24
Age (years), mean ± SD	64.1 ± 9.2	66.7 ± 7.6	67.4 ± 8.4	67.6 ± 7.6
Visual acuity, mean ± SD	0.99 ± 0.05	0.91 ± 0.18*	0.85 ± 0.24*	0.69 ± 0.3**
IOP (mmHg), mean ± SD	17.9 ± 3.5	17.4 ± 3.3	18.6 ± 6.2	23.2 ± 11.9*
CCT (μm), mean ± SD	543.0 ± 32.2	541.5 ± 24.3	537.1 ± 32.5	510.4 ± 34.5**

IOP – intraocular pressure; CCT – central corneal thickness; SD – standard deviation.
Statistical analysis included Kruskal Wallis test: *$p < 0.05$ vs. other groups; **$p < 0.01$ vs. other groups.

Analysis of the OCT parameters of OD and macula are shown in Tables 2 and 3. All examined OCT parameters, including average, superior and inferior RNFL thickness, were significantly lower in patients with glaucoma. Also, the glaucoma patients in the third stage had a substantial decrease in all values compared to the first stage. The C/D area was significantly higher in glaucoma patients and, among them, it was significantly higher in patients with the third stage of glaucoma, compared to other stages.

All OCT measurements of the macula were significantly higher in the healthy subjects compared to glaucoma patients. GCC differed significantly between stages, being highest in the first and lowest in the third stage (Table 3).

Analysis of the OCTA at the level of macula and OD are shown in Tables 4 and 5. All examined OCTA

Table 2

Optical coherence tomography measurement of optic disc

Parameter	Healthy subjects	Patients with glaucoma		
		first stage	second stage	third stage
RNFL (μm), mean ± SD	100.7 ± 6.9*	87.6 ± 10.2	84.2 ± 10.3	67.9 ± 14.2†
RNFL sup. (μm), mean ± SD	103.2 ± 6.7*	89.5 ± 9.7	86.4 ± 10.4	70.3 ± 16.5†
RNFL inf. (μm), mean ± SD	98.3 ± 7.8*	85.9 ± 11.2	81.4 ± 11.7	66.2 ± 15.2†
C/D area (mm^2), mean ± SD	0.34 ± 0.1*	0.44 ± 0.1	0.55 ± 0.2	0.72 ± 0.1#

RNFL –retinal nerve fiber layer; sup. – superior; inf. – inferior; C/D – Cup/Disc area; SD – standard deviation.
Statistical analysis included Kruskal Wallis test or ANOVA: *$p < 0.01$ vs. all stages glaucoma; #$p < 0.01$ vs. other glaucoma stages; †$p < 0.01$ vs. first stage (*post hoc* analysis).

Table 3

Optical coherence tomography measurement of macula

Parameter	Healthy subjects	Patients with glaucoma		
		first stage	second stage	third stage
GCC (μm), mean ± SD	96.3 ± 5.2*	89.5 ± 8.8#	84.9 ± 10.6#	69.8 ± 12.8
GCC sup (μm), mean ± SD	95.6 ± 5.2*	89.5 ± 8.7#	85.2 ± 11.0#	69.8 ± 14.2
GCC inf (μm), mean ± SD	97.1 ± 5.4*	89.6 ± 9.1#	84.7 ± 12.3#	69.3 ± 15.2

GCC – ganglion cell complex; sup. – superior; inf. – inferior.
Statistical analysis included Kruskal Wallis test or ANOVA: *$p < 0.01$ vs. all stages glaucoma; #$p < 0.01$ vs. other glaucoma stages (*post hoc* analysis).

Table 4

Optical coherence tomography angiography measurement of small vessels in optic disc

Parameter	Healthy subjects	Patients with glaucoma		
		first stage	second stage	third stage
WI VCD, mean ± SD	51.03 ± 1.89*	47.18 ± 3.24	46.28 ± 3.44	38.08 ± 6.42#
ID VCD, mean ± SD	51.45 ± 5.08*	46.70 ± 6.46	47.57 ± 5.78	43.36 ± 8.45#
PP VCD, mean ± SD	53.62 ± 2.26*	50.12 ± 3.87	48.59 ± 3.91#	38.04 ± 8.45#
PP sup.VCD, mean ± SD	54.08 ± 2.55*	50.09 ± 3.73	48.96 ± 3.85	38.74 ± 9.17#
PP inf. VCD, mean ± SD	53.36 ± 2.27*	50.05 ± 4.45	48.09 ± 5.05	37.64 ± 9.11#

WI– whole image; VCD – vessel capillary density; ID – inside disc; PP – peripapillary; sup. – superior; inf. – inferior.
Statistical analysis included Kruskal Wallis test or ANOVA: *$p < 0.01$ vs. glaucoma; #$p < 0.01$ vs. other glaucoma stages (*post hoc* analysis).

Table 5

Optical coherence tomography angiography measurement of macula

Parameter	Healthy subjects	Patients with glaucoma		
		first stage	second stage	third stage
FAZ area (mm^2), mean ± SD	0.24 ± 0.09*	0.33 ± 0.14	0.34 ± 0.11	0.35 ± 0.12
FAZ perimeter (mm), mean ± SD	1.99 ± 0.46*	2.33 ± 0.87	2.38 ± 0.53	2.58 ± 0.78
FD (%), mean ± SD	51.46 ± 4.06*	45.29 ± 8.34	46.02 ± 6.97	45.53 ± 6.14
VD SL (%), mean ± SD	47.38 ± 3.24*	41.51 ± 6.39	42.03 ± 4.82	37.44 ± 6.08#
VD SL sup. (%), mean ± SD	47.64 ± 3.77*	40.58 ± 5.99	41.96 ± 4.95	37.66 ± 6.14#
VD SL inf. (%), mean ± SD	47.36 ± 3.42*	41.17 ± 5.87	41.75 ± 5.19	36.90 ± 6.57#
VD DL (%), mean ± SD	52.89 ± 3.25*	50.08 ± 4.71	48.46 ± 5.14	46.23 ± 6.00†
VD DL sup. (%), mean ± SD	53.11 ± 3.17*	50.19 ± 4.85	48.68 ± 5.34	46.86 ± 6.03†
VD DL inf. (%), mean ± SD	52.66 ± 3.58*	50.01 ± 5.00	48.13 ± 5.64	45.78 ± 6.61†

FAZ – foveal avascular zone; FD – vessel density of the 300 μ width ring surrounding the FAZ; VD – macular vessel density in the superficial (SL) and deep (DL) retinal vascular plexus; sup. – superior; inf. – inferior.
Statistical analysis included Kruskal Wallis test or ANOVA:*$p < 0.01$ vs. glaucoma; #$p < 0.01$ vs. other glaucoma stages; †$p < 0.01$ vs. first stage (*post hoc* analysis).

parameters were significantly higher in healthy subjects. All variables were notably lower in patients with the third stage of glaucoma compared to the first stage. Value of peripapillary (PP) VCD differed substantially between all stages, being highest in the first and lowest in the third stage (Table 4).

All examined OCTA parameters (FAZ area and perimeter, FD, VD SL and VD DL) were significantly higher in healthy subjects compared to glaucoma. All VD in superficial layers were substantially lower in the third stage compared to the first and second stages. VD in deep layer was significantly lower in patients with the third stage of glaucoma only compared to the first stage (Table 5).

Pearson correlation coefficient showed significant positive correlation between GCC and VD in superficial retinal vascular plexus, while other OCT and OCTA parameters did not show significant interrelation. Contrary, in glaucoma patients, significant correlation was shown between VD in the superficial and deep retinal vascular plexuses with GCC (Table 6).

The Pearson's correlation coefficient showed significant positive correlation only between RNFL average and whole image VD ($r = 0.314$, $p < 0.05$) in OD of healthy subjects. All OCT and OCTA parameters showed strong and significant positive correlation in patients with glaucoma (Table 7).

Association of OCT and OCTA measurements of macula and OD with presence of glaucoma were analyzed by binary logistic regression model (Enter model). For the binary logistic regression dependent variable was coded as 0-no glaucoma and 1-presence of glaucoma, while independent variables were OCTA and OCT measurements of macula or OD.

The analysis showed significant association of ID VCD – inside disc vessel capillary density with presence of glaucoma [ExpB 0.66; 95% confidence interval (CI) 0.46–0.93)]. The increase of this OCTA parameter for one unit reduced odd for glaucoma appearance for 34%. All other parameters of OD did not show significant association with glaucoma. Other performed analysis of macula parameters showed significant association of OCT parameters: GCC superior (ExpB 0.51; 95% CI 0.29–0.90) and GCC inferior (ExpB 0.46; 95% CI 0.24–0.85). Increasing these parameters for one unit reduced the odds for glaucoma for about 50%. OCTA showed significant association of FD with glaucoma (ExpB 0.76; 95% CI 0.63–0.91) and reduction of odd for 24% for one unit increase.

Discussion

Long-standing mechanical approach to glaucomatous OD damage blames increased intraocular pressure as the main reason for visual impairment. With the introduction of novel techniques capable of measuring vascular density and blood flow, a new vascular theory in the pathogenesis of glaucoma was revealed. One of these techniques, the OCTA,

Table 6

Pearson's correlation coefficient between optical coherence tomography (OCT) and OCT angiography (OCTA) parameters of macula

OCT parameters	OCTA parameters	Healthy subjects	Glaucoma patients
GCC total	FAZ area	-0.257	-0.108
	FAZ perimeter	-0.225	-0.136
	FD	0.241	0.016
	VD SL	0.491**	0.449**
	VD DL	0.226	0.394**
GCC superior	VD SL superior	0.577**	0.390**
	VD DL superior	0.223	0.322**
GCC inferior	VD SL inferior	0.289*	0.538**
GCC inferior	VD DL inferior	0.166	0.222**

GCC – ganglion cell complex; FAZ – foveal avascular zone; FD – vessel density of the 300 µ width ring surrounding the FAZ; VD – vessel density; DL – deep layer; SL –superficial layer.
***p* < 0.05; ****p* < 0.01.**

Table 7

Pearson's correlation coefficient between optical coherence tomography (OCT) and OCT angiography (OCTA) parameters of optical disc

OCT parameters	OCTA parameters	Healthy subjects	Glaucoma patients
RNFL average	WI VCD	0.284	0.698**
	ID VCD	0.276	0.165*
	PP VCD	0.136	0.719**
	WI VD all vessels	0.314*	0.644**
	ID VD all vessels	0.248	0.293**
	PP VD all vessels	0.200	0.727**
RNFL superior	PP VCD superior	0.169	0.664**
RNFL superior	PP VCD superior all vessels	0.134	0.688**
RNFL inferior	PP VCD inferior	0.123	0.707**
RNFL inferior	PP VD inferior all vessels	0.237	0.719**

RNFL –retinal nerve fiber layer; WI – whole image; VCD – vessel capillary density; ID – inside disc; PP – peripapillary; WI – whole *en face* image; VD – vessel density.
***p* < 0.05; ****p* < 0.01.**

is a noninvasive, high-resolution depiction of the vascular structure in the retina. With this as a measurement, the Fechtner and Weinreb [13] theory was established, arguing that the change in blood flow in OD is the pathogenic factor leading to visual field loss, and that macular involvement had significant influence in glaucoma pathogenesis. In this prospective study, we evaluated the characteristics of OCT and OCTA findings in healthy and OAG eyes.

This study confirms the finding of other authors that corneal thickness and IOP play an important part in the diagnosis and understanding of various types of glaucoma [14]. In patients with POAG, all examined OCT parameters in OD and macula differ significantly from those found in healthy subjects and among different stages of POAG. This clearly indicates structural damage in glaucomatous eyes registered by the OCT. Glaucoma leads to progressive damage of retinal ganglion cells and their axons in the RNFL presented with thinning. Clinical follow-up and evaluation of glaucoma needs sensitive methods for detecting progression and preventing visual field loss. OCT and OCTA enable multiple parameters to be used for monitoring disease progression [15]. However, predicting this disease progression, especially in advanced stages of POAG, is challenging because of the existence of a so-called "floor effect", after which no further structural change can be detected in OCT-based RNFL thickness measurements and due to an increase in variability of VF measurements [16]. RNFL in whole and both superior and inferior position was significantly lower in glaucomatous eyes. Literature data indicate that RNFL thinning and rate of average RNFL loss detected by OCT were connected with visual field loss. This stands for both glaucoma suspects and glaucomatous eyes [17, 18]. In line with these results, examined glaucomatous eyes had significantly deteriorated VA and parameters of visual field. Previous studies have already established regional correlations between RNFL loss, GCC loss, and visual field deficits in glaucomatous eyes, showing concomitant structural damages in macula as seen in OD [19].

The importance of the spatial structure of RNFL thickness map data over conventional average circumpapillary RNFL thickness in diagnosing glaucoma was clearly demonstrated in a *post hoc* study of 93 eyes from Los Angeles Latino Eye Study (LALES). Superior diagnostic performance was shown for all models using full RNFL thickness maps [20]. In patients with glaucoma, all sectors and average RNFL showed significant association with the development of glaucoma. In developed glaucomatous eyes, standard determination of RNFL has high clinical value but its determination is primarily connected with prediction of visual field loss.

All examined OCTA parameters at the level of OD and macula showed significant differences in healthy subjects and patients with glaucoma, indicating presence of vascular damage, alongside structural impairment. This is in line with the data from a similar study confirming that VCD is diminished in glaucomatous eyes [21–23]. Some literature data present that all the OD VD parameters except the inside disc VD were significantly lower in glaucomatous eyes than in control eyes [24]. Other results indicate the VCD in the radial papillary capillaries layer and in the nerve head layer of glaucomatous eyes was significantly lower than in age-matched control eyes [1]. The same authors presented results that VCD in all peripapillary segments was higher than in NHL, similarly to our findings.

All examined macular OCTA parameters in superficial and deep layers differentiated in glaucoma presence and the stage of the disease. This highlights a role of vascular factors in the pathogenesis of glaucoma. In a prospective study examining change rate of GCC thickness and macular VD in healthy and OAG eyes, scholars presented results that glaucomatous eyes showed a faster decrease in macular VD than GCC thinning. Faster macular VD decrease rate was significantly associated with the severity of glaucoma, however, the association between GCC thinning rate and glaucoma severity was insignificant [25]. Recent longitudinal study found that rate of change in macula whole *en face* VD was substantially quicker in OPG (-2.23% per year) than in healthy eyes (0.29% *per* year). Conversely, the rate of change in GCC thickness was not significantly different from zero, and no significant differences in the rate of GCC change among diagnostic groups were found [16]. This indicates that vascular changes in macula have a better predictive role for development of OAG than structural changes. Literature data showed that projection-resolved OCTA algorithm, used to remove flow projection artifacts, and superficial vascular complex density in macula, showed high accuracy in detection of glaucoma and glaucoma stages. This could be useful in the clinical evaluation [9]. OCTA could be a promising tool for monitoring glaucoma progression, particularly for patients with advanced glaucoma. This is consistent with a previous study showing that OCTA measurement does not have a detectable floor in the glaucoma continuum, whereas GCC thickness reached the estimated floor at a late stage [26].

To assess a linear correlation between several variables measured by OCT and OCTA in the glaucomatous and healthy eyes, we used the Pearson's correlation. In healthy eyes, there was no correlation between structural changes in OD detected by OCT and vascular changes detected by OCTA. But, in patients with glaucoma, VCD and VD in all examined segments showed a significant and strong positive correlation with RNFL measurements. A similar study demonstrated that peripapillary VCD but not inside disc, had strong correlation with the severity of glaucoma, determined by the measurement of the RNFL [27]. This is also confirmed in the study by Lommatzsch et al. [1] where papillary VCD showed high correlation with RNFL average and rim area.

Macular GCC correlated only with superficial VD in healthy eyes. Contrary, in glaucoma patients, GCC correlated with the thickness of superficial and deep retinal vascular plexuses. The results did not reveal significant correlation between structural and vascular parameters in healthy eyes, but strong positive correlation of all those parameters in glaucomatous eyes indicating close connection between vascular and structural changes. This implicates the importance of vascular disorders in development and progression of glaucoma. In line with this are results that

compared with age-matched control subjects, vascular density of the parafoveal retina decreased in the OAG subjects [28]. It remains unclear whether the reduced microcirculation in glaucoma patients induces the neuronal damage or arises through reduced circulation requirements in damaged tissue. Due to cross-sectional design of the study, we could not conclude if vascular changes precede papillary disc damage, but regression analysis gives us some insight into this question.

This study found that VD in macula and OD predict the development of glaucoma. In a group model, inside disc capillary density and VD of ring surrounding the FAZ showed significant association with presence of glaucoma. OCTA measurements in macula and OD and OCT measurments in macula can predict glaucoma presence, however this is not found in OCT analysis of OD. According to this, it can be assumed that vascular changes in different regions of the retina could be a better predictor for glaucoma development than structural changes.

Conclusion

The analysis of OCT bands and OCTA vascular plexuses may be complementary for the noninvasive quantification of the structural and vascular changes in OD and macula, and accurately distinguishes between healthy and diseased eyes, showing association with presence and development of POAG. Our study demonstrated that reduced VD in glaucomatous eyes correlated with functional and structural changes at the OD and macula in glaucoma. Vascular parameters could be a useful adjunct tool to evaluate/diagnose glaucoma.

R E F E R E N C E S

1. *Lommatzsch C, Rothaus K, Koch JM, Heinz C, Grisanti S.* Vessel density in OCT angiography permits differentiation between normal and glaucomatous optic nerve heads. Int J Ophthalmol 2018; 11(5): 835–43.
2. *Van Melkebeke L, Barbosa-Breda J, Huygens M, Stalmans I.* Optical Coherence Tomography Angiography in Glaucoma: A Review. Ophthalmic Res 2018; 60(3): 139–51.
3. *Lee EJ, Lee KM, Lee SH, Kim T-W.* OCT Angiography of the Peripapillary Retina in Primary Open-Angle Glaucoma. Invest Ophthalmol Vis Sci 2016; 57(14): 6265–70.
4. *Kawasaki R, Wang JJ, Rochtchina E, Lee AJ, Wong TY, Mitchell P.* Retinal Vessel Caliber Is Associated with the 10-year Incidence of Glaucoma. Ophthalmology 2013; 120(1): 84–90.
5. *De Leon JM, Cheung CY, Wong T-Y, Li X, Hamzah H, Aung T,* et al. Retinal vascular caliber between eyes with asymmetric glaucoma. Graefes Arch Clin Exp Ophthalmol 2015; 253(4): 583–9.
6. *Chan KKW, Tang F, Tham CCY, Young AL, Cheung CY.* Retinal vasculature in glaucoma: a review. BMJ Open Ophthalmol 2017; 1(1): e000032.
7. *Holló G.* Optical Coherence Tomography Angiography in Glaucoma. Turk J Ophthalmol 2018; 48(4): 196–201.
8. *Akagi T, Zangwill LM, Shoji T, Suh MH, Saunders LJ, Yarmohammadi A,* et al. Optic disc microvasculature dropout in primary open-angle glaucoma measured with optical coherence tomography angiography. PLoS One 2018; 13(8): e0201729.
9. *Takusagawa HL, Liu L, Ma KN, Jia Y, Gao SS, Zhang M,* et al. Projection-Resolved Optical Coherence Tomography Angiography of Macular Retinal Circulation in Glaucoma. Ophthalmology 2017; 124(11): 1589–99.
10. *Yu J, Gu R, Zong Y, Xu H, Wang X, Sun X,* et al. Relationship Between Retinal Perfusion and Retinal Thickness in Healthy Subjects: An Optical Coherence Tomography Angiography Study. Invest Ophthalmol Vis Sci 2016; 57(9): OCT204-10.
11. *Brusini P.* OCT Glaucoma Staging System: a new method for retinal nerve fiber layer damage classification using spectral-domain OCT. Eye (Lond) 2018; 32(1): 113–9.
12. *Chen CL, Zhang A, Bojikian KD, Wen JC, Zhang Q, Xin C,* et al. Peripapillary Retinal Nerve Fiber Layer Vascular Microcirculation in Glaucoma Using Optical Coherence Tomography–Based Microangiography. Invest Ophthalmol Vis Sci 2016; 57(9): OCT475-85.
13. *Fechtner RD, Weinreb RN.* Mechanisms of optic nerve damage in primary open angle glaucoma. Surv Ophthalmol 1994; 39(1): 23–42.
14. *Bechmann M, Thiel MJ, Roesen B, Ullrich S, Ulbig MW, Ludwig K.* Central corneal thickness determined with optical coherence tomography in various types of glaucoma. Br J Ophthalmol 2000; 84(11): 1233–7.
15. *Dong ZM, Wollstein G, Schuman JS.* Clinical Utility of Optical Coherence Tomography in Glaucoma. Invest Ophthalmol Vis Sci 2016; 57(9): OCT556–67.
16. *Moghimi S, Bowd C, Zangwill LM, Penteado RC, Hasenstab K, Hou H.* Measurement Floors and Dynamic Ranges of OCT and OCT Angiography in Glaucoma. Ophthalmology 2019; 126(7): 980–8.
17. *Niles PI, Greenfield DS, Sehi M, Bhardwaj N, Iverson SM, Chung YS.* Advanced Imaging in Glaucoma Study Group. Detection of progressive macular thickness loss using optical coherence tomography in glaucoma suspect and glaucomatous eyes. Eye (Lond) 2012; 26(7): 983–91.
18. *Miki A, Medeiros FA, Weinreb RN, Jain S, He F, Sharpsten L,* et al. Rates of retinal nerve fiber layer thinning in glaucoma suspect eyes. Ophthalmology 2014; 121(7): 1350–8.
19. *Le PV, Tan O, Chopra V, Francis BA, Ragab O, Varma R,* et al. Regional correlation among ganglion cell complex, nerve fiber layer, and visual field loss in glaucoma. Invest Ophthalmol Vis Sci 2013; 54(6): 4287–95.
20. *An G, Omodaka K, Hashimoto K, Tsuda S, Shiga Y, Takada N,* et al. Glaucoma Diagnosis with Machine Learning Based on Optical Coherence Tomography and Color Fundus Images. J Healthc Eng 2019; 2019: 4061313.
21. *Shin JW, Lee J, Kwon J, Choi J, Kook MS.* Regional vascular density-visual field sensitivity relationship in glaucoma according to disease severity. Br J Ophthalmol 2017; 101(12): 1666–72.
22. *Rao HL, Kadambi SV, Weinreb RN, Puttaiah NK, Pradhan ZS, Rao DA, et al.* Diagnostic ability of peripapillary vessel density measurements of optical coherence tomography angiography in primary open-angle and angle-closure glaucoma. Br J Ophthalmol 2017; 101(8): 1066–70.
23. *Suh MH, Zangwill LM, Manalastas PI, Belghith A, Yarmohammadi A, Medeiros FA,* et al. Optical coherence tomography angiography vessel density in glaucomatous eyes with focal lamina cribrosa defects. Ophthalmology 2016; 123(11): 2309–17.
24. *Hou TY, Kuang TM, Ko YC, Chang YF, Liu CJ, Chen MJ.* Optic Disc and Macular Vessel Density Measured by Optical Coherence Tomography Angiography in Open-Angle and Angle-Closure Glaucoma. Sci Rep 2020; 10(1): 5608.
25. *Hou H, Moghimi S, Proudfoot JA, Ghahari E, Penteado RC, Bowd C,* et al. Ganglion Cell Complex Thickness and Macular Vessel

Density Loss in Primary Open-Angle Glaucoma. Ophthalmology 2020; 127(8): 1043–52.

26. *Shoji T, Zangwill LM, Akagi T, Saunders LJ, Yarmohammadi A, Manalastas PI,* et al. Progressive Macula Vessel Density Loss in Primary Open-Angle Glaucoma: A Longitudinal Study. Am J Ophthalmol 2017; 182: 107–17.
27. *Chen CL, Bojikian KD, Gupta D, Wen JC, Zhang Q, Xin ,* et al. Optic nerve head perfusion in normal eyes and eyes with glaucoma using optical coherence tomography-based microangiography. Quant Imaging Med Surg 2016; 6(2): 125–33.
28. *Tao A, Liang Y, Chen J, Hu H, Huang Q, Zheng J,* et al Structure-function correlation of localized visual field defects and macular microvascular damage in primary open-angle glaucoma. Microvasc Res 2020; 130: 104005.

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