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Optic nerve head diurnal vessel density variations in glaucoma and ocular hypertension measured by optical coherence tomography angiography

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Abstract

Background/aims To evaluate diurnal variations in optic nerve head (ONH) vessel density assessed by optical coherence tomography angiography (OCT-A) in healthy subjects, ocular hypertension (OHT), and open-angle glaucoma (OAG) patients.

Methods Forty subjects (OAG, 21; OHT, 6; healthy, 13) were assessed for vessel density percentage (VD%) and flow index in the ONH (NH VD%, NH index), and in the radial peripapillary capillary layer (RPC VD%, RPC index) at 9:00, 11:00, 14:00, 16:00, and 18:00 on a single day. Repeated measures ANOVAs were used to test for changes in the parameters measured at multiple time points.

Results All OCT-A parameters analyzed at the different time points were statistically lower in the OAG patients compared to both the OHT and healthy groups ($p < 0.05$). In the OAG group, the NH index, RPC index, NH VD%, and RPC VD% were statistically lower at 18:00 compared to 14:00, and the RPC VD% was statistically lower at 9:00 than 14:00. In the OHT group, the RPC index was statistically lower at 9:00 than 11:00. In the healthy group, the NH VD% and RPC VD% were statistically lower at 16:00 than 18:00, and the RPC index was statistically lower at 9:00 than 11:00. No other statistically significant difference was found in none of the three groups comparing any other time point ($p > 0.05$).

Conclusion In healthy subjects, OHT and OAG patients, the variations in the OCT-A derived parameters were relatively small. These results suggest that in the clinical practice the OCT-A assessment can be performed independently of the time of the day, contrasting IOP evaluation.

Keywords Glaucoma · Imaging · Optic nerve

Introduction

Open-angle glaucoma (OAG) represents the leading cause of irreversible blindness worldwide [1]. Elevated intraocular pressure (IOP) has been shown to be associated with the prevalence, incidence, and progression of the disease [2]. Nonetheless, it is well established that glaucoma progression is still observed in some patients with IOP reduction, suggesting that OAG is a multifactorial pathology. Several studies with various imaging modalities, such as color Doppler imaging and confocal scanning laser Doppler flowmetry, have revealed deficiencies of ocular blood flow in the retinal [3], choroidal [4], and retrobulbar circulations [5, 6] in OAG patients.

Optical coherence tomography angiography (OCT-A) is a retinal vascular imaging technology that exploits a novel algorithm to generate high-resolution images and quantify

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vessel density (VD), retinal and choroidal blood flow [6–14]. OCT-A technology has been recently utilized to investigate the vascular pathophysiology of OAG and several studies have demonstrated decreased vessel density in OAG patients with different stages of the disease [6–8, 10–14].

The diurnal variations of IOP, blood pressure (BP), and ocular perfusion pressure (OPP), and the relevance of the fluctuations of these parameters in the progression of glaucoma have been investigated in multiple studies [15–26]. Specifically, several studies evaluating systolic and diastolic blood pressure (SBP and DBP) over a 24-h period showed increased values upon wakening in the morning [16–18] and during periods of wakefulness compared to during sleep [17]. Other studies observed that the IOP peaks upon wakening [19–22] and during the afternoon [19, 20], and it is lowest at midnight [22]. Importantly, Nau et al. [23] showed that the IOP circadian variation is mainly due to the position of the patient (supine or sitting) and that it disappears if patients are measured at all times in one position. Liu et al. showed that, in eyes with early glaucomatous changes compared to healthy eyes diurnal IOP is higher, diurnal-to-nocturnal change of habitual IOP is less, and a different posture-independent IOP pattern around normal awakening time is present [24]. Finally, it has been shown that OPP peaks in the morning and late afternoon or early evening [25, 26], with a trough between 10:00 am and 2:00 pm [26], and that overall mean OPP was lower in the primary OAG patients compared to the glaucoma suspects [26].

The diurnal variation of ocular blood flow assessed with different techniques has shown to demonstrate contrasting results [27–31], and there has recently been an emphasis on the need for more studies investigating the diurnal variations of OCT-A data and their association with IOP changes [32]. The aim of our study was to investigate the diurnal fluctuations of the OCT-A derived parameters in the optic nerve head (ONH) and in the radial peripapillary capillary (RPC) layer in patients with OAG and OHT, and in healthy subjects. In addition, we aimed to evaluate the correlation between the OCT-A parameters measured at different time points and the IOP, OPP, BP, perimetric and structural parameters. The test-retest repeatability of the OCT-A data was also calculated in a subgroup of OAG patients and healthy subjects (supplementary material).

Materials and methods

Forty subjects (OAG: 21; OHT: 6: healthy: 13) were enrolled at the Glaucoma Unit, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), Fondazione G.B. Bietti, Rome, Italy. All patients signed an informed consent prior to initiation of this study, which adhered to the tenets of the Declaration of Helsinki. The study protocol was approved by the Ethical Committee of the IRCCS, Fondazione G.B. Bietti, Rome, Italy (Number/ID of the Approval: 67/18FB). Only male and female subjects older than 18 years of age with the ability to understand and sign the written informed consent were enrolled.

Inclusion criteria for the glaucoma group included the following: history of IOP ≥ 22 mmHg on at least two occasions, open-angle visibility on gonioscopy, the presence of a repeatable visual field (VF) defect (as defined below) corresponding with ONH and peripapillary retinal nerve fiber layer (RNFL) damage as evaluated ophthalmoscopically by two independent expert investigators (F.O. and G.R.), and glaucoma confirmed by the presence of one or more corresponding peripapillary RNFL sectors with thickness outside normal limits assessed by spectral domain OCT (RTVue XR Avanti SD-OCT, Optovue, Fremont, CA, USA).

A glaucomatous VF loss was defined as two consecutive, reliable visual fields with glaucoma hemifield test outside normal limits, mean deviation (MD) and pattern standard deviation (PSD) with $p < 0.05$, and a cluster in the pattern standard deviation plot of at least three points with $p < 0.05$ (one of each with $p < 0.01$) neither contiguous with the blind spot nor crossing the horizontal midline [33]. The reliability indices considered were as follows: false positive < 15%, fixation losses, and false-negative responses < 25%.

OHT subjects required an IOP greater than or equal to 22 mmHg in at least two occasions with no functional and structural evidence of glaucomatous damage while healthy controls had to have an IOP ≤ 22 mmHg, visible open angle on gonioscopy, and normal optic disc and VF test.

Both OAG and OHT patients were included if their IOP was under control (IOP ≤ 22 mmHg) with hypotensive medical treatments (prostaglandin analogues, carbonic anhydrase inhibitors, beta-blockers, in monotherapy or combination).

Participants were excluded for the following reasons: spherical refractive error greater than ± 6 diopters, astigmatism greater than ± 3 diopters, retinal diseases including diabetic retinopathy or age-related macular degeneration, secondary glaucoma (pigmentary glaucoma, exfoliative glaucoma, steroid-induced glaucoma), other optic neuropathies different than glaucoma, opacities of optic media that could bias functional and structural testing, active inflammatory or infectious diseases; metabolic, autoimmune, neurological, or neurodegenerative diseases; cataract surgery within the past 6 months; previous surgical intervention for glaucoma; blood coagulation diseases; pregnancy, or breastfeeding.

All patients were questioned for their demographics, clinical history, ophthalmic history and medications, and systemic diseases and medications. Each subject was evaluated for heart rate (HR) and BP, which was assessed using an automated ambulatory blood pressure monitor after 5 min of rest (systolic blood pressure: SBP; diastolic blood pressure: DBP; mean arterial pressure was calculated as MAP = [(2 x

DBP) + SBP]/3) [34]. A comprehensive ophthalmological examination was performed including slit lamp evaluation, gonioscopy, central corneal thickness and axial length measurements, IOP measurement using Goldmann applanation tonometry, and indirect dilated ophthalmoscopy with a 90 diopters lens. OPP was calculated by the formula OPP = [(2/3 MAP) − IOP]) [34].

Visual function was assessed by standard automated perimetry with the Humphrey field analyzer II using the 24-2 Swedish interactive threshold algorithm standard (white III stimulus) V.4.1 (Carl Zeiss Mediatec, Dublin, CA, USA).

OCT-A imaging was performed using the RTVue XR Avanti SD-OCT with AngioVue software (Optovue, Fremont, CA, USA). This technology uses the algorithm termed split-spectrum amplitude-decorrelation angiography (SSADA) to image the ONH microcirculation. The details of the technique have been described elsewhere [35].

Only images with optimal image quality (signal strength index > 50) were included in the analysis. For the analysis, we used a manufacturer-provided analysis software which automatically provides separate vessel density analysis in the ONH and peripapillary area in two different layers, the "nerve head layer" and the "radial peripapillary capillary (RPC) layer." The nerve head layer extends from the internal limiting membrane (ILM) to 150 μm below the ILM, and the RPC layer extends from the ILM to the outer limit of the RNFL. The OCT-A parameters evaluated in the three groups of subjects were: NH VD% (percentage area occupied by the blood vessels assessed in the optic nerve head layer), RPC VD% (percentage area occupied by the blood vessels assessed in the radial peripapillary capillary layer), NH index (average flow signal assessed in the optic nerve head layer), and RPC index (average flow signal assessed in the radial peripapillary capillary layer). The peripapillary region was defined as a 0.75-mm-wide elliptical annulus extending from the optic disc boundary. For each subject, a single operator acquired two OCT-A scans in order to calculate the test-retest repeatability. In addition to the measurement of the OCT-A parameters, all participants were assessed for ONH structural parameters (cup (C)/disc (D) area ratio, C/D vertical ratio, C/D horizontal ratio, rim area, disc area, cup volume) and thickness of the peripapillary RNFL (average, superior, and inferior) and macular ganglion cell complex (GCC: ganglion cell layer + inner plexiform layer + retinal nerve fiber layer; total, inferior, superior; global loss volume, focal loss volume).

HR, BP, IOP, OPP, and OCT-A measurements were performed at 9:00, 11:00, 14:00, 16:00, and 18:00 time points on a single day with the subjects in the sitting position. All the subjects rested for 30 min before scanning session in order to avoid effects of physical activity on the measurements.

Table 1 Demographic characteristics, systemic parameters, and topical and systemic treatment of open-angle glaucoma (OAG) patients, ocular hypertension (OHT) patients, and healthy subjects. AxL, axial length measured in mm; BB, beta-blockers; CAI: carbonic anhydrase inhibitors; HR, heart rate measured in beats per minute (bpm); MAP, mean arterial pressure measured in mmHg; PAD, diastolic arterial pressure measured in mmHg; PAS, systolic arterial pressure measured in mmHg; OPP, ocular perfusion pressure measured in mmHg; PG, prostaglandin analogues; SD: standard deviation

		OAG *n* (percentage)	OHT *n* (percentage)	Healthy *n* (percentage)
Study eye	Left	18 (56%)	6 (60%)	12 (48%)
	Right	14 (44%)	4 (40%)	13 (52%)
Sex	Female	14 (67%)	5 (83%)	7 (54%)
	Male	7 (33%)	1 (17%)	6 (46%)
Age (years)	Mean (SD), range	65.9 (9.8), 40–77	67 (9.4), 51–77	60.9 (11.9), 33–73
HR (bpm)	Mean (SD)	71.2 (7.9)	67.3 (11.5)	68.5 (5.5)
PAS (mmHg)	Mean (SD)	130.3 (17.8)	139.2 (19.1)	128.7 (18.2)
PAD (mmHg)	Mean (SD)	80.8 (9.0)	79.7 (8.2)	80.2 (10.4)
MAP (mmHg)	Mean (SD)	97.3 (11.5)	99.5 (9.3)	96.4 (11.8)
AxL (mm)	Mean (SD)	24.0 (1.2)	24.6 (1.4)	23.9 (1.1)
OPP (mmHg)	Mean (SD)	81.4 (11.9)	80.8 (10.9)	82.4 (11.3)
Systemic treatment (arterial hypertension)	BB	2 (9.5)	1(16.7)	2 (15.4)
	Calcium-channel blockers	3 (14.3)	0 (0)	1 (7.7)
	Angiotensin II receptor blockers	4 (19)	1 (16.7)	2 (15.4)
Ocular treatment	PG	9 (42.9)	4 (66.7)	0 (0)
	BB	0 (0)	2 (33.3)	0 (0)
	PG+BB	8 (38.1)	0 (0)	0 (0)
	PG+BB+CAI	4 (19)	0 (0)	0 (0)

Statistical analysis

Comparisons among the three study groups for differences in subject-level characteristics were made using chi-square tests and one-way ANOVAs for categorical and continuous variables, respectively. For parameters measured in both eyes, ANOVAs with a fixed group effect and random subject effect were used to compare the groups. Repeated measures ANOVAs were used to test for changes in the parameters that were measured at multiple time points. Pearson correlation coefficients were calculated to assess the linear associations of the OCT-A parameters with systemic and ocular parameters for all subjects combined and by group. The intraclass correlation coefficient (ICC) and the coefficient of variation (%) were used to describe the test-retest repeatability analysis. A 5% significance level was used for all tests.

Results

All forty subjects (OAG: 21; OHT: 6; healthy: 13) completed the study. The overall mean age was 66 years (standard deviation, SD: 10.6). Twenty-six (65%) were female. The three groups did not statistically differ in regard to age, gender, study eye, average HR, SAP, DAP, MAP, OPP, or axial length ($p > 0.05$, Table 1). All the patients were from European descent. The time elapsed since the diagnosis was 5.2 ± 4.3 years for the OAG patients and 3.7 ± 3.3 years for the OHT subjects. Table 1 shows OAG and OHT patients ocular hypotensive and systemic treatment for arterial hypertension in the three subject groups (9/21 OAG patients; 2/6 OHT subjects; 5/13 healthy subjects). No other systemic diseases were reported.

Table 2 displays the means and SD for the ocular parameters in the three studied groups. Based on the Hoddap-Parrish-

Table 2 Ocular parameters of open-angle glaucoma (OAG) patients, ocular hypertension (OHT) patients, and healthy subjects. C, cup; D, disc; GCC thickness, ganglion cell complex (ganglion cell layer + inner plexiform layer + retinal nerve fiber layer) thickness measured in μm; IOP, intraocular pressure; MD, mean deviation measured in decibels (dB); NH, optic nerve head layer; NH index, average flow signal assessed in the optic nerve head layer; NH VD%, percentage area occupied by the blood vessels assessed in the optic nerve head layer; ONH cup volume, optic nerve head cup volume measured in mm^3; ONH disc area, optic nerve head disc area measured in mm^2; ONH rim area, optic nerve head rim area measured in mm^2; PSD, pattern standard deviation measured in decibels (dB); RNFL thickness, retinal nerve fiber layer thickness measured in μm; RPC, radial peripapillary capillary layer; RPC index, average flow signal assessed in the optic nerve head layer; RPC VD%, percentage area occupied by the blood vessels assessed in the radial peripapillary capillary layer. *A p value < 0.05 was considered statistically significant. **When the overall test showed no significant difference among the three groups, the p values for the individual comparisons between groups are not listed

	OAG mean (SD)	OHT mean (SD)	Healthy mean (SD)	Overall *p* value	OAG vs. OHT *p* value	OAG vs. Healthy *p* value	OHT vs. Healthy *p* value
MD (dB)	− 8.5 (5.6)	− 0.2 (0.7)	− 0.6 (1.7)	< 0.001*	< 0.001*	< 0.001*	0.792
PSD (dB)	8.2 (4.1)	1.7 (0.3)	1.9 (0.6)	< 0.001*	< 0.001*	< 0.001*	0.882
IOP average (mmHg)	15.7 (3.7)	19.8 (2.7)	14.5 (2.7)	0.006*	0.007*	0.291	0.002*
RNFL thickness average (μm)	68.2 (9.9)	88.9 (7.7)	96.9 (9.1)	< 0.001*	< 0.001*	< 0.001*	0.100
RNFL thickness superior (μm)	71.0 (13.2)	89.1 (7.2)	98.8 (9.0)	< 0.001*	0.001*	< 0.001*	0.098
RNFL thickness inferior (μm)	65.6 (8.9)	88.6 (8.8)	95.0 (10.6)	< 0.001*	< 0.001*	< 0.001*	0.188
ONH C/D area ratio	0.70 (0.13)	0.35 (0.32)	0.32 (0.20)	< 0.001*	< 0.001*	< 0.001*	0.667
ONH C/D vertical ratio	0.85 (0.08)	0.47 (0.35)	0.50 (0.21)	< 0.001*	< 0.001*	< 0.001*	0.793
ONH C/D horizontal ratio	0.88 (0.11)	0.54 (0.39)	0.56 (0.23)	< 0.001*	0.002*	< 0.001*	0.861
ONH rim area (mm^2)	0.59 (0.23)	1.07 (0.33)	1.24 (0.35)	< 0.001*	0.001*	< 0.001*	0.194
ONH disc area (mm^2)	2.04 (0.37)	1.86 (0.48)	1.99 (0.52)	0.555	**	**	**
ONH cup volume (mm^3)	0.51 (0.31)	0.19 (0.21)	0.15 (0.19)	< 0.001*	0.008*	< 0.001*	0.692
GCC thickness total (μm)	72.0 (9.3)	90.2 (8.1)	94.1 (7.1)	< 0.001*	< 0.001*	< 0.001*	0.393
GCC thickness superior (μm)	75.5 (11.2)	90.2 (8.1)	93.9 (7.2)	< 0.001*	0.002*	< 0.001*	0.576
GCC thickness inferior (μm)	68.8 (12.1)	90.1 (8.6)	94.5 (7.4)	< 0.001*	< 0.001*	< 0.001*	0.308
GCC focal loss volume (%)	8.1 (5.2)	0.8 (1.0)	0.9 (1.4)	< 0.001*	< 0.001*	< 0.001*	0.974
GCC global loss volume (%)	24.3 (8.6)	6.9 (5.3)	3.9 (4.4)	< 0.001*	< 0.001*	< 0.001*	0.321
NH index	0.080 (0.012)	0.095 (0.016)	0.095 (0.016)	0.005*	0.023*	0.003*	0.945
RPC index	0.045 (0.011)	0.062 (0.017)	0.064 (0.015)	< 0.001*	0.008*	< 0.001*	0.767
NH VD%	74.1 (9.2)	86.4 (8.0)	85.3 (8.7)	0.001*	0.005*	0.001*	0.896
RPC VD%	44.2 (10.5)	62.3 (13.9)	63.1 (11.7)	< 0.001*	0.002*	< 0.001*	0.848

Anderson classification, 9 OAG patients had an early defect (MD between 0 dB and − 6 dB), 6 had a moderate defect (MD between − 6 dB and − 12 dB), and 6 had a severe defect (MD greater than − 12 dB). A statistically significant difference among the three groups was found in: IOP average, perimetric global indices (MD and PSD), RNFL

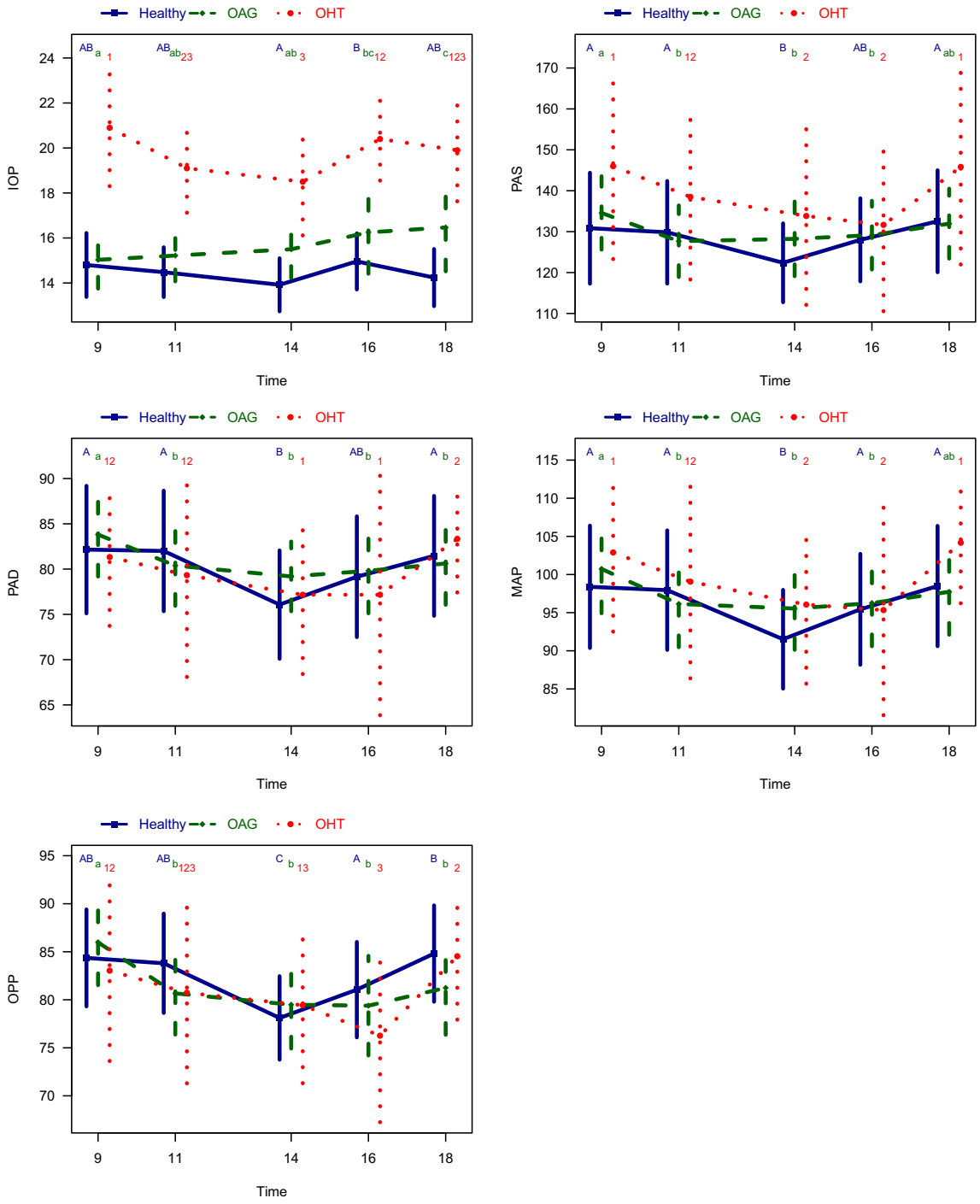

Fig. 1 Plots of the ocular and systemic parameters (top left: IOP, intraocular pressure measured in mmHg; top right: PAS, systolic arterial pressure measured in mmHg; middle left: PAD, diastolic arterial pressure measured in mmHg; middle right: MAP, mean arterial pressure measured in mmHg; bottom: OPP, ocular perfusion pressure measured in mmHg) measured at different time points (9:00, 11:00, 14:00, 16:00, and 18:00) in open-angle glaucoma (OAG) patients (dashed green line), ocular hypertension (OHT) patients (dotted red line), and healthy subjects (solid blue line). Mean with 95% confidence interval. Significant differences between groups ($p < 0.05$) are represented by non-overlapping confidence intervals. For comparisons between time points within groups, times with the same uppercase letters are not different in the healthy patients, times with the same lowercase letters are not different in the OAG patients, times with the same numbers are not different in the OHT patients

thickness (average, superior, inferior), ONH parameters (C/D area ratio, C/D vertical ratio, C/D horizontal ratio, rim area, cup volume), and GCC (thickness total, superior and inferior; focal and global loss volume) (all p values < 0.05, Table 2). Only IOP was different between OHT patients and healthy subjects ($p = 0.002$, Table 2); all other significant differences were among the OAG patients and the other two groups.

Figure 1 and Table 3 present the means and SD for IOP, BP (MAP, PAS, PAD), and OPP measured at different time points (9:00, 11:00, 14:00, 16:00, and 18:00) in the three different groups.

Figure 2 and Table 4 present the means and SD for OCT-A parameters measured at different time points (9:00, 11:00, 14:00, 16:00, and 18:00), as well as the average values in the three studied groups and the differences between time points for the IOP and OCT-A measurements are shown. A statistically significant difference was found among the three groups both for the average OCT-A derived parameters and at

Table 3 Systemic and ocular parameters (means and standard deviations, SD) measured at different time points (9:00, 11:00, 14:00, 16:00, and 18:00) in open-angle glaucoma (OAG) patients, ocular hypertension (OHT) patients, and healthy subjects. P_1: comparison p value between OAG and OHT patients; P_2: comparison p value between OAG patients and healthy subjects; P_3: comparison p value between OHT patients and healthy subjects. IOP, intraocular pressure measured in mmHg; MAP, mean arterial pressure measured in mmHg; PAD, diastolic arterial pressure measured in mmHg; PAS, systolic arterial pressure measured in mmHg; OPP, ocular perfusion pressure measured in mmHg; SD: standard deviation. *A p value < 0.05 was considered statistically significant. **When the overall test showed no significant difference among the three groups, the p values for the individual comparisons between groups are not listed

Measurement	Time	OAG (SD)	OHT (SD)	Healthy (SD)	p value ($p < 0.05$) Overall comparison	Comparison p values		
						P_1 OAG vs OHT p value	P_2 OAG vs healthy p value	P_3 OHT vs healthy p value
IOP	9:00	15.0 (3.6)	20.9 (3.6)	14.8 (3.4)	0.001*	0.001*	0.852	0.001*
	11:00	15.2 (3.2)	19.1 (2.8)	14.5 (2.7)	0.005*	0.005*	0.404	0.002*
	14:00	15.5 (3.5)	18.5 (3.3)	13.9 (2.9)	0.020*	0.035*	0.213	0.006*
	16:00	16.3 (5.1)	20.4 (2.6)	15.0 (3.0)	0.032*	0.032*	0.368	0.010*
	18:00	16.5 (5.4)	19.9 (3.2)	14.2 (3.1)	0.042*	0.109	0.142	0.013*
	Average	15.7 (3.7)	19.8 (2.7)	14.5 (2.7)	0.006*	0.007*	0.291	0.002*
PAS	9:00	134.6 (19.7)	146.0 (21.7)	130.8 (22.4)	0.346	**	**	**
	11:00	127.7 (19.2)	138.5 (19.3)	129.8 (20.7)	0.501	**	**	**
	14:00	128.2 (20.0)	133.8 (20.7)	122.4 (16.0)	0.447	**	**	**
	16:00	129.1 (18.5)	131.7 (20.1)	128.0 (16.8)	0.920	**	**	**
	18:00	132.0 (18.8)	145.8 (22.7)	132.5 (20.6)	0.313	**	**	**
	Average	130.3 (17.8)	139.2 (19.1)	128.7 (18.2)	0.489	**	**	**
PAD	9:00	83.8 (10.2)	81.3 (7.3)	82.2 (11.7)	0.833	**	**	**
	11:00	80.4 (9.7)	79.3 (10.7)	82.0 (11.0)	0.848	**	**	**
	14:00	79.2 (8.5)	77.2 (8.4)	76.1 (9.9)	0.605	**	**	**
	16:00	79.8 (10.3)	77.2 (12.7)	79.2 (11.0)	0.872	**	**	**
	18:00	80.6 (10.0)	83.3 (5.6)	81.5 (11.0)	0.837	**	**	**
	Average	80.8 (9.0)	79.7 (8.2)	80.2 (10.4)	0.963	**	**	**
MAP	9:00	100.7 (12.8)	102.9 (9.9)	98.4 (13.3)	0.750	**	**	**
	11:00	96.1 (12.5)	99.1 (12.1)	97.9 (13.0)	0.851	**	**	**
	14:00	95.5 (11.9)	96.1 (9.9)	91.5 (10.7)	0.555	**	**	**
	16:00	96.3 (12.4)	95.3 (13.1)	95.4 (12.0)	0.977	**	**	**
	18:00	97.7 (12.4)	104.2 (7.6)	98.5 (13.1)	0.514	**	**	**
	Average	97.3 (11.5)	99.5 (9.3)	96.4 (11.8)	0.854	**	**	**
OPP	9:00	86.0 (12.4)	83.0 (13.2)	84.4 (12.2)	0.736	**	**	**
	11:00	80.7 (11.9)	80.7 (13.2)	83.8 (12.5)	0.763	**	**	**
	14:00	79.5 (12.7)	79.4 (11.4)	78.1 (10.5)	0.826	**	**	**
	16:00	79.4 (14.4)	76.3 (12.6)	81.1 (12.0)	0.657	**	**	**
	18:00	81.2 (13.6)	84.5 (9.2)	84.8 (12.1)	0.793	**	**	**
	Average	81.4 (11.9)	80.8 (10.9)	82.4 (11.3)	0.906	**	**	**

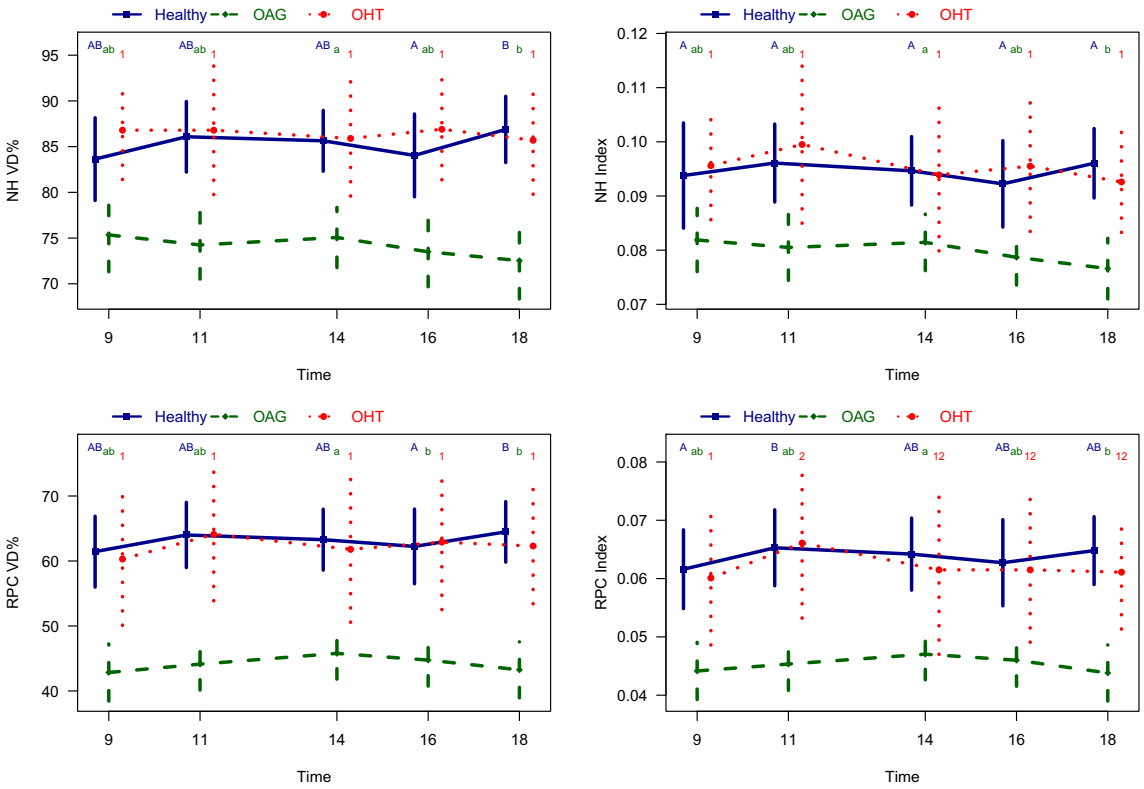

Fig. 2 Plots of the optical coherence tomography angiography parameters (top left: NH VD%, percentage area occupied by the blood vessels in the optic nerve head layer; bottom left: RPC VD%, percentage area occupied by the blood vessels in the radial peripapillary capillary layer; top right: NH index, average flow signal assessed in the optic nerve head layer; bottom right: RPC index, average flow signal assessed in the radial peripapillary capillary layer) measured at different time points (9:00, 11:00, 14:00, 16:00, and 18:00) in open-angle glaucoma (OAG) patients (dashed green line), ocular hypertension (OHT) patients (dotted red line), and healthy subjects (solid blue line). Mean with 95% confidence interval. Significant differences between groups ($p < 0.05$) are represented by non-overlapping confidence intervals. For comparisons between time points within groups, times with the same uppercase letters are not different in the healthy patients, times with the same lowercase letters are not different in the OAG patients, times with the same numbers are not different in the OHT patients

all varying time points ($p < 0.05$) except the NH index measured at 9:00 ($p = 0.079$, Table 4). Similar to above, differences were present among the OAG patients and the other two groups; OHT and healthy patients were not significantly different for any of the OCT-A parameters. In the OAG group, the NH index, RPC index, NH VD%, and RPC VD% were statistically higher at 14:00 compared to 18:00, and the RPC VD% was statistically lower at 9:00 than 14:00, whereas all other time points did not statistically differ ($p > 0.05$, Table 4). In the OHT group, the RPC index was statistically lower at 9:00 than 11:00, and all other time points did not statistically differ ($p > 0.05$, Table 4). In the healthy group, the NH VD% and RPC VD% were statistically lower at 16:00 than 18:00, and the RPC index was statistically lower at 9:00 than 11:00, while no other statistically significant difference was found comparing any other time point ($p > 0.05$, Table 4).

In Table 5, the correlation coefficients between the systemic, ocular, and average OCT-A derived parameters are indicated for the three groups. For all study subjects combined, moderate to strong statistically significant correlations ($|r| > 0.5$, all $p < 0.001$) were found for NH index, RPC index, NH VD%, and RPC VD% with RNFL thickness average/superior/inferior, ONH C/D area ratio, ONH C/D vertical and horizontal ratios, ONH rim area, GCC thickness total/superior/inferior, and GCC global loss volume as well as for RPC index, NH VD%, and RPC VD% with MD and ONH cup volume. Figure 3 shows several representative plots.

In Supplementary Table 1, the test-retest repeatability of the OCT-A data is indicated for the group of OAG patients and healthy subjects.

Discussion

The OAG group displayed statistically lower MD, higher PSD, structural damage of the ONH, along with decreased peripapillary RNFL and GCC thickness compared to the healthy and OHT groups (Table 2, p values < 0.001). These results agree with previous studies showing that glaucomatous damage is characterized by ONH morphological changes and

Table 4 Optical coherence tomography angiography parameters (means and standard deviations, SD) measured at different time points (9:00, 11:00, 14:00, 16:00, and 18:00) in open-angle glaucoma (OAG) patients, ocular hypertension (OHT) patients, and healthy subjects. Time comparisons by group (OAG patients, OHT patients, healthy subjects) for intraocular pressure (IOP) and optical coherence tomography angiography parameters measured at different time points (9:00, 11:00, 14:00, 16:00, and 18:00). P_1: comparison p value between OAG and OHT patients; P_2: comparison p value between OAG patients and healthy subjects; P_3: comparison p value between OHT patients and healthy subjects. NH: optic nerve head layer; NH index: average flow signal assessed in the optic nerve head layer; NH VD%: percentage area occupied by the blood vessels assessed in the optic nerve head layer; RPC: radial peripapillary capillary layer; RPC index: average flow signal assessed in the radial peripapillary capillary layer; RPC VD%: percentage area occupied by the blood vessels in the radial peripapillary capillary layer. *A p value < 0.05 was considered statistically significant. **When the overall test showed no significant difference among the three groups, the p values for the individual comparisons between groups are not listed

Measurement	Time	OAG (SD)	OHT (SD)	Healthy (SD)	p-value ($p < 0.05$) Overall comparison	Comparison p-values		
						P_1	P_2	P_3
NH index	9:00	0.082 (0.016)	0.096 (0.014)	0.094 (0.024)	0.079	**	**	**
	11:00	0.081 (0.017)	0.100 (0.020)	0.096 (0.017)	0.010*	0.020*	0.008*	0.769
	14:00	0.081 (0.014)	0.094 (0.020)	0.095 (0.015)	0.032*	0.096	0.014*	0.812
	16:00	0.079 (0.014)	0.096 (0.017)	0.092 (0.019)	0.013*	0.024*	0.011*	0.749
	18:00	0.077 (0.016)	0.093 (0.013)	0.096 (0.016)	0.001*	0.021*	< 0.001*	0.535
	Average	0.080 (0.012)	0.095 (0.016)	0.095 (0.016)	0.005*	0.023*	0.003*	0.945
RPC index	9:00	0.044 (0.014)	0.060 (0.016)	0.062 (0.016)	0.002*	0.020*	0.001*	0.784
	11:00	0.045 (0.013)	0.066 (0.018)	0.065 (0.016)	< 0.001*	0.003*	< 0.001*	0.940
	14:00	0.047 (0.012)	0.062 (0.020)	0.064 (0.015)	0.003*	0.027*	0.001*	0.694
	16:00	0.046 (0.012)	0.062 (0.017)	0.063 (0.018)	0.004*	0.024*	0.002*	0.859
	18:00	0.044 (0.013)	0.061 (0.014)	0.065 (0.014)	< 0.001*	0.007*	< 0.001*	0.547
	Average	0.045 (0.011)	0.062 (0.017)	0.064 (0.015)	< 0.001*	0.008*	< 0.001*	0.767
NH VD%	9:00	75.3 (11.2)	86.8 (7.6)	83.6 (11.0)	0.013*	0.017*	0.014*	0.577
	11:00	74.3 (10.4)	86.8 (9.9)	86.1 (9.3)	0.002*	0.010*	0.001*	0.992
	14:00	75.1 (9.1)	85.9 (8.8)	85.6 (8.1)	0.001*	0.010*	0.001*	0.959
	16:00	73.5 (10.6)	86.9 (7.7)	84.0 (11.0)	0.002*	0.004*	0.002*	0.570
	18:00	72.5 (11.6)	85.7 (8.3)	86.9 (8.8)	< 0.001*	0.005*	< 0.001*	0.720
	Average	74.1 (9.2)	86.4 (8.0)	85.3 (8.7)	0.001*	0.005*	0.001*	0.896
RPC VD%	9:00	42.8 (12.2)	60.3 (14.3)	61.4 (13.2)	< 0.001*	0.005*	< 0.001*	0.801
	11:00	44.1 (11.1)	64.1 (14.3)	64.0 (12.2)	< 0.001*	0.001*	< 0.001*	0.951
	14:00	45.8 (11.0)	61.8 (15.7)	63.3 (11.4)	< 0.001*	0.005*	< 0.001*	0.804
	16:00	44.8 (11.1)	62.9 (14.5)	62.2 (13.9)	< 0.001*	0.003*	< 0.001*	0.911
	18:00	43.3 (12.0)	62.3 (12.4)	64.5 (11.3)	< 0.001*	0.001*	< 0.001*	0.652
	Average	44.2 (10.5)	62.3 (13.9)	63.1 (11.7)	< 0.001*	0.002*	< 0.001*	0.848

Measurement	Time comparison	OAG		OHT		Healthy	
		Result	p-value	Result	p-value	Result	p-value
IOP	9 vs. 11	9 & 11	0.587	9 > 11	0.004*	9 & 11	0.413
	9 vs. 14	9 & 14	0.300	9 > 14	0.003*	9 & 14	0.086
	9 vs. 16	9 < 16	0.042*	9 & 16	0.640	9 & 16	0.813
	9 vs. 18	9 < 18	0.024*	9 & 18	0.379	9 & 18	0.436
	11 vs. 14	11 & 14	0.496	11 & 14	0.417	11 & 14	0.232
	11 vs. 16	11 & 16	0.076	11 & 16	0.211	11 & 16	0.465
	11 vs. 18	11 < 18	0.034*	11 & 18	0.446	11 & 18	0.718
	14 vs. 16	14 & 16	0.109	14 < 16	0.024*	14 < 16	0.050*
	14 vs. 18	14 < 18	0.040*	14 & 18	0.096	14 & 18	0.547
	16 vs. 18	16 & 18	0.510	16 & 18	0.400	16 & 18	0.056
NH index	9 vs. 11	9 & 11	0.603	9 & 11	0.409	9 & 11	0.442
	9 vs. 14	9 & 14	0.887	9 & 14	0.758	9 & 14	0.805
	9 vs. 16	9 & 16	0.227	9 & 16	0.983	9 & 16	0.606

Table 4 (continued)

Measurement	Time	OAG (SD)	OHT (SD)	Healthy (SD)	*p*-value ($p < 0.05$) Overall comparison	Comparison *p*-values		
						P_1	P_2	P_3
	9 vs. 18		9 & 18	0.064	9 & 18	0.555	9 & 18	0.482
	11 vs. 14		11 & 14	0.708	11 & 14	0.211	11 & 14	0.611
	11 vs. 16		11 & 16	0.423	11 & 16	0.323	11 & 16	0.134
	11 vs. 18		11 & 18	0.100	11 & 18	0.104	11 & 18	0.988
	14 vs. 16		14 & 16	0.208	14 & 16	0.682	14 & 16	0.332
	14 vs. 18		14 > 18	0.049*	14 & 18	0.767	14 & 18	0.614
	16 vs. 18		16 & 18	0.232	16 & 18	0.354	16 & 18	0.056
RPC index	9 vs. 11		9 & 11	0.468	9 < 11	0.041*	9 < 11	0.048*
	9 vs. 14		9 & 14	0.141	9 & 14	0.688	9 & 14	0.239
	9 vs. 16		9 & 16	0.277	9 & 16	0.644	9 & 16	0.559
	9 vs. 18		9 & 18	0.851	9 & 18	0.760	9 & 18	0.124
	11 vs. 14		11 & 14	0.287	11 & 14	0.105	11 & 14	0.547
	11 vs. 16		11 & 16	0.659	11 & 16	0.085	11 & 16	0.129
	11 vs. 18		11 & 18	0.350	11 & 18	0.088	11 & 18	0.795
	14 vs. 16		14 & 16	0.501	14 & 16	1.000	14 & 16	0.393
	14 vs. 18		14 > 18	0.040*	14 & 18	0.886	14 & 18	0.734
	16 vs. 18		16 & 18	0.102	16 & 18	0.867	16 & 18	0.169
NH VD%	9 vs. 11		9 & 11	0.410	9 & 11	1.000	9 & 11	0.105
	9 vs. 14		9 & 14	0.852	9 & 14	0.739	9 & 14	0.243
	9 vs. 16		9 & 16	0.180	9 & 16	0.968	9 & 16	0.797
	9 vs. 18		9 & 18	0.059	9 & 18	0.678	9 & 18	0.054
	11 vs. 14		11 & 14	0.507	11 & 14	0.681	11 & 14	0.751
	11 vs. 16		11 & 16	0.547	11 & 16	0.964	11 & 16	0.148
	11 vs. 18		11 & 18	0.202	11 & 18	0.647	11 & 18	0.599
	14 vs. 16		14 & 16	0.187	14 & 16	0.637	14 & 16	0.233
	14 vs. 18		14 > 18	0.048*	14 & 18	0.930	14 & 18	0.390
	16 vs. 18		16 & 18	0.350	16 & 18	0.517	16 < 18	0.016*
RPC VD%	9 vs. 11		9 & 11	0.293	9 & 11	0.082	9 & 11	0.064
	9 vs. 14		9 < 14	0.041*	9 & 14	0.559	9 & 14	0.258
	9 vs. 16		9 & 16	0.152	9 & 16	0.274	9 & 16	0.594
	9 vs. 18		9 & 18	0.782	9 & 18	0.446	9 & 18	0.067
	11 vs. 14		11 & 14	0.148	11 & 14	0.261	11 & 14	0.578
	11 vs. 16		11 & 16	0.572	11 & 16	0.545	11 & 16	0.161
	11 vs. 18		11 & 18	0.469	11 & 18	0.405	11 & 18	0.725
	14 vs 16		14 & 16	0.365	14 & 16	0.589	14 & 16	0.419
	14 vs 18		14 > 18	0.027*	14 & 18	0.806	14 & 18	0.351
	16 vs 18		16 & 18	0.128	16 & 18	0.733	16 < 18	0.045*

thinning of the peripapillary RNFL and macular GCC, which result in corresponding perimetric defects [36]. As it is shown in Table 2, the VD values in the radial peripapillary layer of the three groups were very high (RPC VD% ranging from 44.2 to 63.1%, Table 2); these results agree with a previous study [29]. A possible explanation of our results may be the multiple layers of capillaries needed to supply the thick RNFL near the optic disc, whereas in most of the area supplied by these capillaries, possibly outside the measurement zone, there would only be one layer. This is supported by the large values for RNFL thickness, which in healthy individuals is close to 100 μm (Table 2).

As shown in Fig. 2 and Table 4, all OCT-A parameters analyzed at the different time points were statistically lower in the OAG patients compared to both the OHT and healthy groups. These results confirmed what has been demonstrated

Table 5 Correlation coefficients of Pearson (R value) between the average optical coherence tomography-angiography (OCT-A) derived parameters and the systemic and ocular measurements in open-angle glaucoma (OAG) patients, ocular hypertension (OHT) patients, and healthy subjects. C, cup; D, disc; GCC, ganglion cell complex; HR, heart rate; IOP, intraocular pressure; MAP, mean arterial pressure; MD, mean deviation; NH, optic nerve head layer; NH index, average flow signal assessed in the optic nerve head layer; NH VD%, percentage area occupied by the blood vessels assessed in the optic nerve head layer; ONH, optic nerve head; OPP, ocular perfusion pressure; PAD, diastolic arterial pressure; PAS, systolic arterial pressure; PSD, pattern standard deviation; RNFL, retinal nerve fiber layer; RPC, radial peripapillary capillary layer; RPC index, average flow signal assessed in the radial peripapillary capillary layer; RPC VD%, percentage area occupied by blood vessels in the radial peripapillary capillary layer. *A p value < 0.05 was considered statistically significant

OCT-A measurement	Comparison	Overall		OAG		OHT		Healthy	
		R value	*p* value	*R* value	*p* value	*R* value	*p* value	*R* value	*p* value
NH index	Systemic parameters								
	HR	0.12	0.473	0.23	0.323	0.80	0.059	− 0.04	0.892
	PAS	− 0.34	0.032*	− 0.24	0.302	− 0.26	0.642	− 0.73	0.003*
	PAD	− 0.14	0.392	− 0.12	0.613	0.55	0.283	− 0.36	0.227
	MAP	− 0.26	0.106	− 0.18	0.429	0.14	0.803	− 0.59	0.033*
	Ocular parameters								
	MD	0.42	< 0.001*	0.17	0.348	0.54	0.107	0.09	0.682
	PSD	− 0.22	0.068	0.41	0.020*	0.10	0.784	− 0.16	0.440
	IOP	− 0.07	0.565	− 0.20	0.271	0.46	0.187	− 0.26	0.214
	OPP	− 0.27	0.028*	− 0.22	0.234	− 0.15	0.683	− 0.49	0.012*
	RNFL thickness average	0.62	< 0.001*	0.63	< 0.001*	0.40	0.260	0.40	0.049*
	RNFL thickness superior	0.60	< 0.001*	0.62	< 0.001*	0.33	0.361	0.34	0.097
	RNFL thickness inferior	0.60	< 0.001*	0.49	0.004*	0.40	0.257	0.41	0.041*
	ONH C/D area ratio	− 0.63	< 0.001*	− 0.25	0.161	− 0.73	0.013*	− 0.50	0.010*
	ONH C/D vertical ratio	− 0.60	< 0.001*	− 0.26	0.148	− 0.65	0.042*	− 0.43	0.031*
	ONH C/D horizontal ratio	− 0.59	< 0.001*	− 0.12	0.504	− 0.65	0.041*	− 0.49	0.011*
	ONH rim area	0.57	< 0.001*	0.30	0.101	0.69	0.027*	0.36	0.077
	ONH disc area	− 0.33	0.006*	0.00	0.981	− 0.70	0.023*	− 0.43	0.031*
	ONH cup volume	− 0.48	< 0.001*	− 0.01	0.972	− 0.78	0.006*	− 0.62	0.001*
	GCC thickness total	0.64	< 0.001*	0.54	0.001*	0.26	0.481	0.62	0.001*
	GCC thickness superior	0.69	< 0.001*	0.68	< 0.001*	0.33	0.366	0.63	0.001*
	GCC thickness inferior	0.52	< 0.001*	0.20	0.285	0.18	0.625	0.54	0.005*
	GCC focal loss volume	− 0.34	0.005*	0.17	0.352	− 0.64	0.046*	− 0.52	0.007*
	GCC global loss volume	− 0.59	< 0.001*	− 0.50	0.003*	− 0.26	0.477	− 0.46	0.021*
RPC index	Systemic parameters								
	HR	0.10	0.542	0.24	0.307	0.69	0.140	0.04	0.893
	PAS	− 0.35	0.027*	− 0.24	0.298	− 0.40	0.457	− 0.79	0.001*
	PAD	− 0.17	0.297	− 0.12	0.609	0.37	0.496	− 0.44	0.138
	MAP	− 0.28	0.079	− 0.19	0.425	− 0.06	0.918	− 0.66	0.012*
	Ocular parameters								
	MD	0.51	< 0.001*	0.24	0.193	0.57	0.083	0.24	0.242
	PSD	− 0.33	0.006*	0.32	0.077	0.22	0.562	− 0.20	0.335
	IOP	− 0.09	0.494	− 0.26	0.147	0.59	0.075	− 0.25	0.226
	OPP	− 0.28	0.022*	− 0.21	0.262	− 0.28	0.453	− 0.57	0.002*
	RNFL thickness average	0.69	< 0.001*	0.58	< 0.001*	0.62	0.054	0.36	0.075
	RNFL thickness superior	0.66	< 0.001*	0.54	0.001*	0.57	0.085	0.34	0.099
	RNFL thickness inferior	0.67	< 0.001*	0.48	0.005*	0.60	0.065	0.36	0.078
	ONH C/D area ratio	− 0.73	< 0.001*	− 0.18	0.317	− 0.84	0.001*	− 0.66	< 0.001*
	ONH C/D vertical ratio	− 0.72	< 0.001*	− 0.26	0.149	− 0.76	0.008*	− 0.60	0.001*
	ONH C/D horizontal ratio	− 0.68	< 0.001*	0.04	0.850	− 0.75	0.009*	− 0.68	< 0.001*
	ONH rim area	0.64	< 0.001*	0.27	0.141	0.79	0.005*	0.37	0.071

Table 5 (continued)

OCT-A measurement	Comparison	Overall		OAG		OHT		Healthy	
		R value	*p* value	*R* value	*p* value	*R* value	*p* value	*R* value	*p* value
	ONH disc area	− 0.38	0.001*	0.04	0.819	− 0.82	0.003*	− 0.57	0.002*
	ONH cup volume	− 0.51	< 0.001*	0.12	0.501	− 0.83	0.002*	− 0.74	< 0.001*
	GCC thickness total	0.72	< 0.001*	0.55	0.001*	0.41	0.252	0.68	< 0.001*
	GCC thickness superior	0.75	< 0.001*	0.65	< 0.001*	0.48	0.166	0.72	< 0.001*
	GCC thickness inferior	0.61	< 0.001*	0.24	0.184	0.32	0.382	0.58	0.002*
	GCC focal loss volume	− 0.39	0.001*	0.21	0.256	− 0.67	0.030*	− 0.56	0.003*
	GCC global loss volume	− 0.67	< 0.001*	− 0.49	0.004*	− 0.40	0.263	− 0.53	0.006*
NH VD%	Systemic parameters								
	HR	0.11	0.517	0.30	0.195	0.67	0.161	− 0.05	0.883
	PAS	− 0.21	0.199	− 0.15	0.518	− 0.01	0.992	− 0.66	0.012*
	PAD	− 0.07	0.688	− 0.02	0.926	0.67	0.158	− 0.33	0.283
	MAP	− 0.15	0.363	− 0.09	0.705	0.39	0.474	− 0.53	0.061
	Ocular parameters								
	MD	0.54	< 0.001*	0.33	0.064	0.55	0.103	0.11	0.597
	PSD	− 0.33	0.007*	0.24	0.188	0.31	0.404	− 0.13	0.535
	IOP	− 0.07	0.579	− 0.14	0.457	0.25	0.491	− 0.31	0.131
	OPP	− 0.16	0.193	− 0.13	0.479	0.07	0.844	− 0.42	0.037*
	RNFL thickness average	0.69	< 0.001*	0.71	< 0.001*	0.46	0.191	0.28	0.170
	RNFL thickness superior	0.68	< 0.001*	0.67	< 0.001*	0.35	0.336	0.27	0.186
	RNFL thickness inferior	0.65	< 0.001*	0.57	< 0.001*	0.49	0.151	0.27	0.192
	ONH C/D area ratio	− 0.69	< 0.001*	− 0.39	0.027*	− 0.82	0.002*	− 0.57	0.003*
	ONH C/D vertical ratio	− 0.66	< 0.001*	− 0.35	0.048*	− 0.75	0.010*	− 0.51	0.008*
	ONH C/D horizontal ratio	− 0.65	< 0.001*	− 0.30	0.091	− 0.76	0.008*	− 0.56	0.003*
	ONH rim area	0.62	< 0.001*	0.36	0.042*	0.76	0.009*	0.36	0.074
	ONH disc area	− 0.39	0.001*	− 0.14	0.432	− 0.77	0.007*	− 0.55	0.004*
	ONH cup volume	− 0.57	< 0.001*	− 0.13	0.495	− 0.88	< 0.001*	− 0.74	< 0.001*
	GCC thickness total	0.72	< 0.001*	0.59	< 0.001*	0.42	0.232	0.62	0.001*
	GCC thickness superior	0.76	< 0.001*	0.70	< 0.001*	0.48	0.172	0.63	< 0.001*
	GCC thickness inferior	0.60	< 0.001*	0.27	0.131	0.35	0.328	0.53	0.006*
	GCC focal loss volume	− 0.43	< 0.001*	0.05	0.768	− 0.70	0.021*	− 0.60	0.001*
	GCC global loss volume	− 0.69	< 0.001*	− 0.56	0.001*	− 0.38	0.295	− 0.47	0.017*
RPC VD%	Systemic parameters								
	HR	0.09	0.595	0.26	0.250	0.68	0.148	0.02	0.947
	PAS	− 0.26	0.109	− 0.18	0.442	− 0.25	0.652	− 0.75	0.002*
	PAD	− 0.12	0.448	− 0.07	0.761	0.45	0.400	− 0.42	0.157
	MAP	− 0.21	0.201	− 0.13	0.581	0.09	0.876	− 0.63	0.019*
	Ocular parameters								
	MD	0.60	< 0.001*	0.36	0.043	0.55	0.101	0.21	0.323
	PSD	− 0.42	< 0.001*	0.22	0.220	0.33	0.359	− 0.16	0.438
	IOP	− 0.07	0.548	− 0.21	0.248	0.49	0.155	− 0.31	0.136
	OPP	− 0.22	0.077	− 0.18	0.323	− 0.16	0.671	− 0.53	0.005*
	RNFL thickness average	0.75	< 0.001*	0.65	< 0.001*	0.68	0.028*	0.35	0.090
	RNFL thickness superior	0.72	< 0.001*	0.60	< 0.001*	0.60	0.065	0.34	0.093
	RNFL thickness inferior	0.73	< 0.001*	0.55	0.001*	0.68	0.027*	0.33	0.113
	ONH C/D area ratio	− 0.79	< 0.001*	− 0.30	0.094	− 0.92	< 0.001*	− 0.68	< 0.001*
	ONH C/D vertical ratio	− 0.76	< 0.001*	− 0.34	0.060	− 0.85	0.001*	− 0.62	0.001*
	ONH C/D horizontal ratio	− 0.73	< 0.001*	− 0.10	0.572	− 0.85	0.001*	− 0.68	< 0.001*

Table 5 (continued)

OCT-A measurement	Comparison	Overall		OAG		OHT		Healthy	
		R value	*p* value	*R* value	*p* value	*R* value	*p* value	*R* value	*p* value
	ONH rim area	0.70	< 0.001*	0.34	0.054	0.85	0.001*	0.39	0.056
	ONH disc area	− 0.40	0.001*	− 0.03	0.860	− 0.88	< 0.001*	− 0.62	0.001*
	ONH cup volume	− 0.57	< 0.001*	0.05	0.794	− 0.90	< 0.001*	− 0.77	< 0.001*
	GCC thickness total	0.79	< 0.001*	0.61	< 0.001*	0.47	0.173	0.69	< 0.001*
	GCC thickness superior	0.80	< 0.001*	0.71	< 0.001*	0.53	0.115	0.72	< 0.001*
	GCC thickness inferior	0.68	< 0.001*	0.29	0.107	0.39	0.271	0.60	0.001*
	GCC focal loss volume	− 0.46	< 0.001*	0.16	0.373	− 0.72	0.017*	− 0.63	< 0.001*
	GCC global loss volume	− 0.74	< 0.001*	− 0.55	0.001*	− 0.46	0.191	− 0.55	0.004*

in several studies that have investigated the changes in the ONH morphology and ocular perfusion by OCT-A in patients with glaucoma at different stages exhibiting a decreased vessel density in glaucomatous patients [7, 8, 11–14, 37]. Collectively, these results highlight the possible role of ONH perfusion impairment in the glaucomatous optic neuropathy and suggest that OCT-A can be used with other imaging techniques for the non-invasive diagnosis and follow-up of patients with OAG and OHT.

Yousefi recently highlighted the importance of studies investigating diurnal variations in OCT-A data [32], since only few studies have been published on this topic [29–31]. To our knowledge, this represents the first study investigating the diurnal variations of OCT-A parameters in OAG patients, healthy controls, and OHT subjects (previous studies investigated biomarkers only in glaucomatous and healthy subjects) [29–31]. In our study, we found that in the OAG group, the NH index, RPC index, NH VD%, and RPC VD% were statistically lower at 18:00 compared to 14:00, and the RPC VD% was statistically lower at 9:00 than 14:00. Likewise, in the OHT group, the RPC index was statistically lower at 9:00 than 11:00. In the healthy group, the NH VD% and RPC VD% were statistically lower at 16:00 than 18:00, and the RPC index was statistically lower at 9:00 than 11:00. No other statistically significant differences were found (Table 4). These results agree with those of Mansouri et al. who also

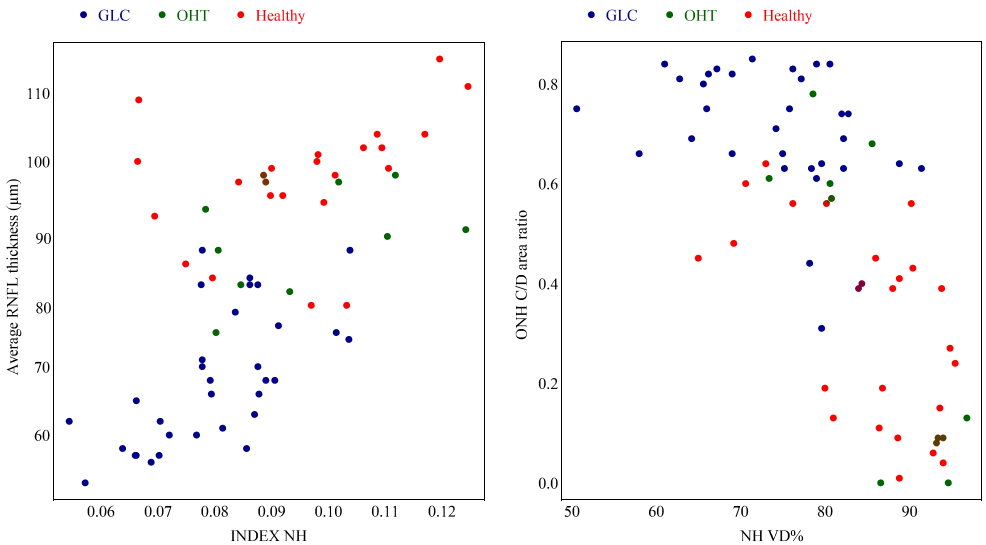

Fig. 3 Plots of NH index vs RNFL thickness average (left) and NH VD% vs ONH C/D area ratio (right), using average of measurements over time within each subject. NH index: average flow signal assessed in the optic nerve head layer; NH VD%: percentage area occupied by the blood vessels in the optic nerve head layer; ONH C/D area ratio: optic nerve head cup/disc area ratio; RNFL thickness: retinal nerve fiber layer thickness measured in µm. Open-angle glaucoma (GLC) patients (blue), ocular hypertension (OHT) patients (green), and healthy subjects (red)

did not find any statistical differences among the evaluated OCT-A parameters except for the ONH and peripapillary VD measurements at the 14:00 and 16:00 time points, which were significantly greater than the measurements at the 08:00 and 11:00 time points [29]. Similarly, in the study conducted by Müller et al., peripapillary flow density assessed in 40 glaucomatous patients was not affected by diurnal changes ($p = 0.319$) [30]. Our results confirm these findings not only in the glaucomatous subjects, but also in the OHT and healthy groups suggesting that, both under pathological and physiological conditions, the vessel density assessed by OCT-A does not present significant diurnal variations. However, contrasting results have been recently published by Baek et al., who found greater diurnal changes in the retinal vessel density in glaucomatous patients compared to healthy subjects [31]. Additional studies are therefore needed in order to elucidate the diurnal variation of OCT-A derived parameters, not only in the peripapillary region, but also at the level of the macula.

In our study, we also evaluated the diurnal variations in IOP, BP, and OPP (Fig. 1, Tables 3 and 4). Interestingly, the 18:00 time point had statistically higher IOP than every other time point for at least one of the study groups. This result differs from a previous study by Liu et al. [24], in which IOP at the 18:00 time point was lower IOP than the earlier time points. The discrepancy with the published literature may be due to methodological differences in the IOP measurement (Goldmann tonometer versus pneumatonometer), and to the effect on IOP of both topical and systemic medications that could have influenced the results differently in different studies.

Finally, we found moderate to strong significant correlations between the OCT-A parameters and the structural measurements (RNFL thickness, ONH parameters, GCC thickness), while no or weak correlation was found with the IOP and systemic parameters (Table 5, Fig. 3). These results confirm those of previous studies [7, 11, 12] and suggest a correlation between vascular damage and neurodegeneration in the pathophysiology of glaucoma.

There are several limitations to this study. First, the timepoints of measures (9:00, 11:00, 14:00, 16:00, and 18:00) were chosen to be representative of office hours during which the measurements are commonly performed such as those by Mansouri et al. [29] but they do not address nocturnal measurement variations. Additional measurement timepoints may reveal a more comprehensive profile of daily OCT-A variations, including 6:00 am which has been suggested to be an important time for assessing clinical parameters such as IOP [38]. It is important to highlight that all the measurements were taken with the subjects in the sitting position. The circadian variation in the IOP parameter has been shown to be mainly due to the position of the patient (supine or sitting) and to disappear if patients are measured at all times in one position [23]. Therefore, the sitting posture of the subjects during the examination may have an effect on our results. Also, we acknowledge that the two subgroups of the healthy and OHT subjects have a small sample size. Our study suggests that there may be no diurnal variation in the evaluated parameters, but given the limitation of the sample size, a larger study is needed in order to confirm our results. The signal strength index (SSI) has been previously shown to have a significant correlation with the vascular density [29]. In our study, only scans with SSI > 50 were used, while it would have been important to include the SSI as a function of time in the statistical analyses; further studies are needed to address this issue. Finally, it should be highlighted that potential confounds that may have affected our results were both the selection of subjects with an IOP under 22 mmHg, and the presence of antiglaucomatous and antihypertensive medications. Specifically, both topical IOP-lowering medications and systemic antihypertensive medications used by study subjects (Table 1) may influence the ocular and systemic vascular systems [39, 40]. Studies involving larger sample of subjects are therefore needed in order to investigate the role of these factors and their relationship with the OCT-A parameters.

In conclusion, for the first time, our study displayed no statistically significant diurnal variation in the ONH ocular blood flow measurements assessed by OCT-A in OAG, OHT, and healthy subjects. Our findings suggest that within the clinical office setting, the OCT-A assessment and the follow-up of the patients does not require repetition of the exam timepoints, contrasting suggestions for exact overlapping IOP evaluations. However, further research is needed in order to evaluate the influence of the topical IOP-lowering medications and systemic antihypertensive drugs on the OCT-A derived parameters throughout the diurnal and nocturnal periods.

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Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

Ethics statement The study protocol was approved by the Ethical Committee of the IRCCS, Fondazione G.B. Bietti, Rome, Italy. All patients signed an informed consent prior to initiation of this study, which adhered to the tenets of the Declaration of Helsinki.

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