Effectiveness of optical coherence tomography angiography (OCT-A) in staging glaucoma

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ABSTRACT

Objectives: To analyze whether vessel density or nerve fiber layer thicknes (structural properties) obtained by optical coherence tomography angiography (OCT-A) could differentiate between healthy controls and patients with ocular hypertension or mild, moderate and severe glaucoma. In addition, to determine if vessel density or nerve fiber layer thickness is most helpful in detecting early glaucomatous damage.

Methods: In this retrospective study, we selected 69 healthy controls, 36 patients with ocular hypertension, and 91 with primary open-angle glaucoma (54 mild, 25 moderate, and 12 severe). One eye was randomly selected per patient. Patients were excluded if they were < 18 years, had secondary glaucoma, OCT-A signal strength index < 40, refractive errors > ± 5 D, vision worse than 20/40, or only one functional eye. Collected data included: age, ethnicity, gender, family history of glaucoma, intraocular pressure, visual fields, cup/disc ratio, and OCT-A macular and optic nerve head scanning parameters.

Results: Whole image optic nerve head and macular vessel density both decreased as the glaucoma progressed, from ocular hypertension to severe stage, 56.7% to 43.1% and 49.0% to 43.4%, respectively ($p<0.01$). Similarly, average nerve fiber layer thickness decreased from, 92.0μm to 60.1μm ($p<0.01$) in patients with ocular hypertension to those with severe glaucoma. Both structural properties and vessel density were equally effective at determining glaucoma stage. Between healthy controls and patients with ocular hypertension, we noticed structural property differences, but no vessel density differences.

Conclusion: Both optic nerve head and macular vessel density and structural properties assessed by OCT-A may provide an objective measure of glaucomatous damage in the eye. In addition, we have found that structural damage may occur before vessel density damage in ocular hypertension.

Keywords: primary open angle glaucoma, optical coherence tomography angiography, ocular hypertension.

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INTRODUCTION

Glaucoma is the second most common cause of blindness worldwide and primary open-angle glaucoma (POAG) is the most prevalent form(1).

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The latter is a chronic, progressive optic neuropathy with a characteristic atrophy of the optic nerve, loss of retinal ganglion cells and their axons, and an open anterior chamber angle with or without elevated intraocular pressure (IOP)(1). Currently, POAG is diagnosed and classified based on visual field (VF) testing using standard automated perimetry(1). However, the VF test is subjective, time consuming, poor at reproducibility, and often is unable to diagnose early glaucoma damage or provide reliable evidence of progression of the disease(2). Due to the limitations of VF testing and the frequent variability of IOP measurements, ophthalmologists cannot always determine whether a patient with ocular hypertension will progress to glaucoma based on VF or IOP alone(1,3). Therefore, there exists a need for new, objective, and reliable methods to both diagnose glaucoma and glaucoma progression early in order to start appropriate treatment.

In recent years, optic coherence tomography (OCT) has provided a non-invasive way to measure macular ganglion cell complex (mGCC) and retinal nerve fiber layer (RNFL) thickness and has been shown to notice glaucomatous damage before VF changes are noted by up to eight years and to determine the glaucoma stage(4,5). Since 2014, OCT-A is a new, non-invasive modality that can in addition visualize the microvasculature of the optic nerve head (ONH), retina, choroid, and peripapillary region and provide reproducible and quantitative vessel density measurements that can help in the management of POAG(6). While previous studies have shown a decrease in vessel density in glaucoma, it is not well known if OCT-A is also effective in detecting early glaucoma damage in ocular hypertension or progression in patients diagnosed with glaucoma(6).

Therefore, this retrospective study was designed to determine if structural or vessel properties measured by OCT-A could differentiate between healthy controls, and those diagnosed with ocular hypertension or POAG (mild, moderate, and severe), and which of these two methods is more helpful in detecting early glaucomatous damage.

METHODS

Study Design

In this retrospective, case-control study, we reviewed the electronic medical records of all consecutive patients diagnosed with ocular hypertension or POAG who underwent OCT-A at the University of Texas Southwestern (UTSW) Medical Center Eye Clinic from March 2016 to June 2017. A cohort of healthy individuals was also selected. A list of all patients undergoing OCT-A was maintained by clinical staff at the UTSW tertiary care eye clinic. Approval by the Institutional Review Board of UTSW was obtained, and we followed the tenets of the United States Health Insurance Portability and Accountability Act of 1996 (HIPAA) and the Declaration of Helsinki for research involving human subjects.

Selection of Patients

All patients who had reliable OCT-A and VF test results and were above 18 years old were included. Patients were excluded if they had secondary glaucoma, narrow angle-closure glaucoma, retinal diseases (diabetic retinopathy, hypertensive retinopathy), history of intraocular surgery other than uncomplicated cataract or glaucoma surgery, uveitis, ocular trauma, Parkinson disease, Alzheimer disease, stroke, VF fixation loss >33%, VF false-positive or false-negative errors >33%, refractive errors greater than ± 5 diopters or greater than ± 3 cylinder, corrected visual acuity worse than 20/40, or only one functional eye. Unreliable OCT-A images were defined as images with an SSI <40, motion artifacts, segmentation errors, or poor clarity.

Patients were divided into 5 separate groups: healthy patients without glaucoma (controls), ocular hypertension, and POAG (mild, moderate, and severe). Healthy controls had IOP less than 21 mmHg bilaterally with no history of elevated IOP, intact neuroretinal rims and retinal nerve fiber layer,

no VF defects, and were not on any anti-glaucoma medications. Patients with ocular hypertension had no VF defects and at least one of the following: IOP ≥ 21 mmHg, suspicious optic discs, or were on anti-glaucoma medications. Patients were diagnosed with POAG if they had suspicious optic discs, open iridocorneal angles confirmed by gonioscopy, and VF defects characteristic of glaucoma. Suspicious optic discs were defined as noticeable cupping, neuroretinal rim thinning or notching, or an RNFL defect characteristic of glaucoma[1]. Patients with POAG were separated into mild, moderate, or severe based on their VF mean deviation (MD) of >-6 dB, between -6 dB and -12 dB, and < -12 dB, respectively, using the modified Hodapp-Parrish-Anderson classification(7).

Data Collection

Systemic patient data collected included: age, ethnicity, gender, diagnosis of systemic hypertension, diabetes, or cataracts, family history of glaucoma, and body mass index (BMI). Ocular measurements collected included: best corrected visual acuity as LogMAR units (BCVA), spherical equivalent, central corneal thickness (CCT) (Cornea-Gage Plus Sonogage Pachymeter; Sonogage, Inc., Cleveland, OH), IOP measured by Goldmann Applanation Tonometer (Haag-Streit, Inc., Koeniz, Switzerland), VF MD and pattern standard deviation (PSD) measured by Humphrey Field Analyzer 3 and analyzed with the Humphrey 24-2 Swedish Interactive Threshold Algorithm (Humphrey Instruments, CA, USA), cup/disc ratio (C/D), previous ocular surgeries, previous laser trabeculoplasties, number of anti-glaucoma medications, and use of systemic carbonic anhydrase medications.

OCT-A operators were blinded to the patient's glaucoma diagnosis. OCT-A data was exported with Optovue RTVue XR version 2016.2.0.35 (Avanti Widefield OCT with AngioVue OCT Angiography; Optovue, Inc., Fremont, CA). The device uses an 840-nm diode laser source and has a scanning rate of 70,000 A-scans per second(8). The software provides vascular data at various retinal layers with an en face angiogram and a quantitative representation of vessel density(9). The device shows the eye's vessel distribution by using split-spectrum amplitude-decorrelation to compare the decorrelation signal, based on the differences in backscattered OCT signal intensity(10). By taking multiple A-scans, the scans are compiled into B-scans, which allow cross-sectional structural information(11). Optic disc vessel density measurement is made by using 3mm x 3mm field of view images centered on the ONH and focusing on the segment from the internal limiting membrane to the posterior boundary of RNFL. Macular vessel density is obtained with a 3mm x 3mm field of view images centered on the macula homing in on the inner limiting membrane to the inner plexiform layer. The peripapillary and parafovea vessel densities were calculated with a 750 μm elliptical margin from the optic disc and an annular margin with an inner diameter of 1 mm and an outer diameter of 2.5 mm from the fovea, respectively(9). Examples of a moderate POAG patient's vessel density en face images are seen in Figure 1.

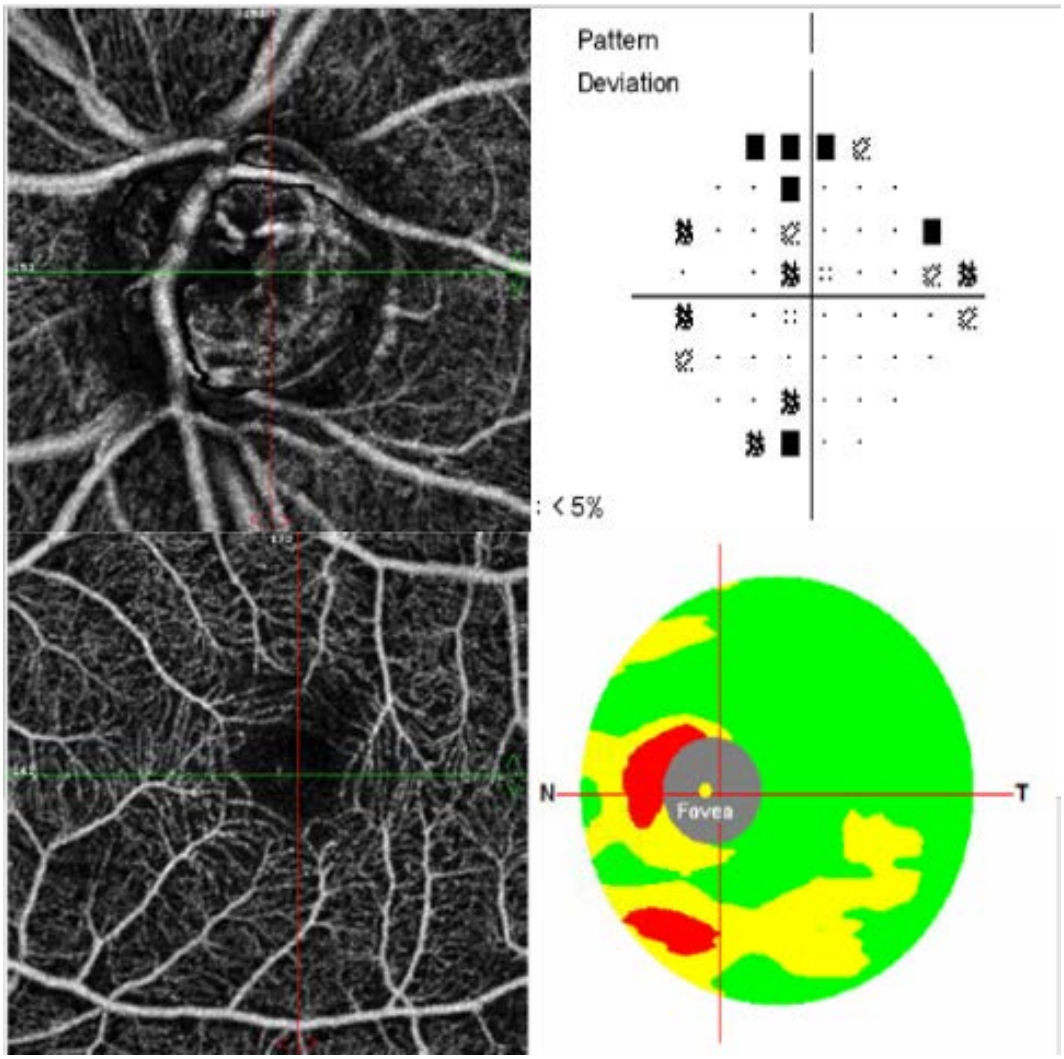


Figure 1: OCT-A and VF images of a patient diagnosed with moderate POAG.

Figure 1:
Top row: Optic nerve head en face angiograph with corresponding pattern standard deviation.
Bottom row: Macular en face angiograph with mGCC map

OCT-A scanning parameters exported were: foveal avascular zone (FAZ) area (mm^2), disc area (mm^2), C/D, horizontal C/D, vertical C/D, cup area (mm^2), rim area (mm^2), rim volume (mm^3), nerve head volume (mm^3), cup volume (mm^3), RNFL SSI, RNFL thickness (µm) (average, superior, inferior, temporal, nasal, superior nasal, superior temporal, inferior nasal, and inferior temporal), mGCC SSI, mGCC thickness (µm) (inner retina, superior, inferior), mGCC focal loss volume (FLV) (%) and global loss volume (GLV) (%), ONH vessel density (%) (SSI, whole image, inside disc, peripapillary, nasal, temporal, superior temporal, superior nasal, inferior temporal, and inferior nasal), and macular vessel density (%) (SSI, whole image, fovea, parafovea, superior, inferior, temporal, and nasal). GLV and FLV are pattern analyses of the mGCC thickness map provided by Optovue(12). GLV represents an average amount of mGCC loss across the entire eye and has the same concept as mean deviation of VF, while FLV represents focal mGCC loss after correcting for the general depression in the mGCC thickness topography map and has the same concept as pattern standard deviation of VF(12).

Statistical Analyses

Statistical analyses of data were performed with IBM SPSS Statistics (IBM SPSS, Inc., New York, NY). One eye was randomly selected per patient through the Microsoft Excel random number function. A chi-square test was used to compare differences between groups of categorical data and Jonckheere-Terpstra trend test and independent samples t-tests were used to compare differences among groups for continuous data. Inter-patient coefficients of variations (CV) were computed to evaluate the relative variability among structural or vessel properties for determining glaucoma stage. The CV is a measure of standard deviation that is normalized to the mean for assessing precision. Multivariable linear regression was used to determine the relationship between VF MD as the dependent variable and the whole image macular vessel density, whole image optic disc vessel density, average RNFL, and average mGCC as the independent variables while controlling for potential confounders, such as age, BMI, gender, ethnicity, family history of glaucoma, presence of hypertension, diabetes, or cataracts, BCVA, and SSI. An alpha level of 0.05 was the cutoff for statistical significance.

RESULTS

Baseline Characteristics

From 383 patients who fit the inclusion criteria, 187 patients (48.8%) were excluded based on our exclusion criteria. Patients were divided into 5 separate groups: 69 healthy controls (patients without glaucoma), 36 patients with ocular hypertension, and 91 with POAG (54 mild, 25 moderate, and 12 severe). Demographic characteristics, structural properties, and vessel densities of healthy control, ocular hypertension and glaucomatous eyes are shown in Table I, Table II, and Table III, respectively.

Table I: Demographic characteristics of healthy controls, ocular hypertension, and glaucomatous eyes

Variables		Controls (n=69) (%)	Ocular Hypertension (n=36) (%)	Mild Glaucoma (n=54) (%)	Moderate Glaucoma (n=25) (%)	Severe Glaucoma (n=12) (%)	P value*- all groups	P value**- controls and ocular hypertension	P value ‡ - ocular hypertension and POAG
Age (years)		60.52±11.67	64.42±10.05	69.19±9.32	69.16±11.12	67.08±11.28	<0.01	0.09	0.14
BMI (kg/m²)		28.83±5.55	28.58±7.52	27.89±6.1	28.3±7.13	30.04±6	0.84	0.85	0.79
Gender	Male	24 (34.8)	11 (30.6)	22 (40.7)	17 (68)	1 (8.3)	<0.01	0.66	<0.01
	Female	45 (65.2)	25 (69.4)	32 (59.3)	8 (32)	11 (91.7)			
Ethnicity	White	30 (43.5)	22 (61.1)	27 (50)	9 (36)	8 (66.7)	0.35	0.25	0.29
	Black	18 (26.1)	9 (25)	17 (31.5)	7 (28)	1 (8.3)			
	Hispanic	8 (11.6)	2 (5.6)	4 (7.4)	6 (24)	1 (8.3)			
	Others	13 (18.8)	3 (8.3)	6 (11.1)	3 (12)	2 (16.7)			
Family History of Glaucoma	Yes	33 (47.8)	22 (61.1)	28 (51.9)	6 (24)	4 (33.3)	0.12	0.16	0.12
	No	32 (46.4)	10 (27.8)	21 (38.9)	14 (56)	6 (50)			

	Unknown	4 (5.8)	4 (11.1)	5 (9.3)	5 (20)	2 (16.7)			
Hypertension	No	35 (50.7)	18 (50)	18 (33.3)	5 (20)	6 (50)	0.04	0.94	0.08
	Yes	34 (49.3)	18 (50)	36 (66.7)	20 (80)	6 (50)			
Diabetes	No	53 (76.8)	28 (77.8)	42 (77.8)	14 (56)	8 (66.7)	0.24	0.91	0.18
	Yes	16 (23.2)	8 (22.2)	12 (22.2)	11 (44)	4 (33.3)			
Hyperopia	No	33 (48.5)	12 (33.3)	26 (48.1)	9 (36)	4 (33.3)	0.13	0.43	0.08
	Mild (<= -2D)	13 (19.1)	9 (25.0)	2 (3.7)	7 (28)	5 (41.7)			
	Moderate (-2D- -3D)	2 (2.9)	1 (2.8)	3 (5.6)	0	0			
	Severe (>=-3D)	0	1 (2.8)	0	0	0			
	Unknown	20 (29.4)	13 (36.1)	23 (42.6)	9 (36)	3 (25)			
Myopia	No	16 (23.5)	12 (33.3)	8 (14.8)	8 (32)	6 (50)	0.36	0.16	0.30
	Mild (<= -2D)	22 (32.4)	5 (13.9)	18 (33.3)	8 (32)	2 (16.7)			
	Moderate (-2D- -3D)	3 (4.4)	3 (8.3)	3 (5.6)	0	1 (8.3)			
	Severe (>=-3D)	6 (8.8)	1 (2.8)	3 (5.6)	1 (4)	0			
	Unknown	21 (30.9)	15 (41.7)	22 (40.7)	8 (32)	3 (25)			
Best Corrected Visual Acuity (LogMAR)	0	46 (66.7)	26 (72.2)	36 (66.7)	9 (36)	6 (50)	0.03	0.95	0.02
	0.1	16 (23.2)	7 (19.4)	14 (25.9)	9 (36)	2 (16.7)			
	0.2	5 (7.2)	2 (5.6)	4 (7.4)	6 (24)	2 (16.7)			
	0.3	2 (2.9)	1 (2.8)	0	1 (4)	2 (16.7)			
Cataract Status	None	19 (27.5)	5 (13.9)	9 (16.7)	3 (12)	1 (8.3)	<0.01	0.14	0.13
	1+	36 (52.2)	20 (55.6)	19 (35.2)	9 (36)	5 (41.7)			
	2+	8 (11.6)	3 (8.3)	8 (14.8)	1 (4)	0			
	3+	0	0	0	2 (8)	0			
	Pseudophakia	6 (8.7)	8 (22.2)	18 (33.3)	10 (40)	6 (50)			
Past Ocular Surgery	No	65 (94.2)	34 (94.4)	43 (79.6)	21 (84)	8 (66.7)	0.02	0.96	0.10
	Yes	4 (5.8)	2 (5.6)	11 (20.4)	4 (16)	4 (33.3)			
Past Laser Trabeculoplasty	No	69 (100)	31 (86.1)	51 (94.4)	21 (84)	10 (83.3)	0.04	<0.01	0.40
	Yes	0	5 (13.9)	3 (5.6)	4 (16)	2 (16.7)			
Number of Ocular Medications	0	69 (100)	3 (8.3)	0	4 (16)	1 (8.3)	<0.01	<0.01	<0.01
	1	0	27 (75)	34 (63)	14 (56)	5 (41.7)			
	2	0	6 (16.7)	18 (33.3)	3 (12)	2 (16.7)			
	3	0	0	2 (3.7)	4 (16)	3 (25)			
	5	0	0	0	0	1 (8.3)			
Systemic Carbonic Anhydrase Inhibitor	No	69 (100)	36 (100)	52 (96.3)	25 (100)	10 (83.3)	<0.01	1.00	0.03
	Yes	0	0	2 (3.7)	0	2 (16.7)			
IOP (mm Hg)		14.94±2.75	15.94±4.64	15.04±3.87	14.76±4.31	13.88±4.4	0.50	0.17	0.46
CCT (μm)		536.13±36.44	563.97±35.90	546.94±32.26	525.29±33.23	548.1±67.89	<0.01	<0.01	<0.01
Visual Field	Mean Deviation	-1.16±1.50	-0.80±1.51	-2.29±2.01	-8.26±1.51	-18.69±4.38	<0.01	0.24	<0.01

	Pattern Standard Deviation	1.91±0.74	1.88±0.97	2.96±1.84	6.9±3.15	11.35±3.31	<0.01	0.86	<0.01

Baseline clinical data of patient eye per group reported as mean ± standard deviation.
P-values from independent t-test and Jonckheere-Terpstra trend for continuous data and chi-square for categorical data
*P-values for all groups
**P-values between controls and patients with ocular hypertension
‡ P-values between patients with ocular hypertension and POAG (mild, moderate, and severe)

Table II: Structural properties of healthy controls, ocular hypertension, and glaucomatous eyes

Variables	Controls (n=69) (%)	Ocular Hypertension (n=36) (%)	Mild Glaucoma (n=54) (%)	Moderate Glaucoma (n=25) (%)	Severe Glaucoma (n=12) (%)	P value*- all groups	P value**- controls and ocular hypertension	P value ‡ - ocular hypertension and POAG
FAZ Area (mm^2)	0.34±0.14	0.34±0.12	0.33±0.14	0.4±0.16	0.36±0.16	0.51	0.95	0.39
Avg SSI RNFL	57.91±12.43	57.07±11.98	55.6±8.58	49.62±12.06	51.55±9.68	0.02	0.75	0.03
Optic Disc Area (mm^2)	2.39±0.45	2.03±0.39	2.26±0.53	2.17±0.55	1.93±0.69	<0.01	<0.01	0.09
C/D ratio	0.49±0.14	0.43±0.17	0.54±0.15	0.57±0.17	0.73±0.12	<0.01	0.05	<0.01
Horizontal C/D ratio	0.77±0.14	0.68±0.2	0.78±0.14	0.81±0.13	0.9±0.1	<0.01	0.01	<0.01
Vertical C/D ratio	0.66±0.13	0.62±0.17	0.73±0.13	0.76±0.14	0.85±0.11	<0.01	0.15	<0.01
Optic Cup Area (mm^2)	1.21±0.46	0.91±0.49	1.25±0.47	1.24±0.47	1.4±0.48	<0.01	0	<0.01
Optic Rim Area (mm^2)	1.18±0.28	1.11±0.29	1.01±0.34	0.93±0.39	0.53±0.35	<0.01	0.26	<0.01
Optic Rim Volume (mm^3)	0.1±0.05	0.1±0.05	0.07±0.05	0.06±0.05	0.02±0.02	<0.01	0.93	<0.01
Nerve Head Volume (mm^3)	0.2±0.1	0.21±0.1	0.15±0.08	0.14±0.09	0.05±0.05	<0.01	0.98	<0.01
Cup Volume (mm^3)	0.43±0.27	0.3±0.31	0.4±0.27	0.36±0.29	0.47±0.27	0.21	0.03	0.27
Average RNFL (µm)	96.11±9.8	92±10.02	82.95±11.75	76.91±14.45	60.13±7.51	<0.01	0.05	<0.01
Superior RNFL (µm)	97.78±10.81	94.54±10.76	85.1±12.51	79.58±13.79	61.27±10.71	<0.01	0.16	<0.01
Inferior RNFL (µm)	94.45±10.32	89.47±10.41	80.8±12.71	74.24±16.29	58.99±7.28	<0.01	0.02	<0.01
Temporal RNFL (µm)	71.15±9.52	70.08±11.26	63.4±9.45	59.49±11.18	50.3±11	<0.01	0.61	<0.01
Nasal RNFL (µm)	74.69±11.07	73.32±11.67	68.27±11.86	62.74±12.01	54.58±12.28	<0.01	0.56	<0.01
RNFL Superior Temporal (µm)	127.73±15.6	123.37±19.67	107.8±17.38	100.68±19.7	71.57±19.16	<0.01	0.23	<0.01

RNFL Superior Nasal (µm)	106.63±18.59	100.63±17.2	92.79±19.5	87.97±19.21	66.46±13.76	<0.01	0.12	<0.01
RNFL Inferior Nasal (µm)	108.71±18.52	102.02±16.76	93.51±19.05	87.64±24.28	66.29±14.12	<0.01	0.08	<0.01
RNFL Inferior Temporal (µm)	134.12±17.8	123.18±19.67	106.18±23.03	94.52±29.81	66.95±12.42	<0.01	0.01	<0.01
Avg SSI Ganglion Cell Complex	65.15±9.44	63.27±10.34	62.11±6.83	59.81±7.76	57.35±8.5	0.01	0.36	0.12
mGCC Average (µm)	94.29±9.65	90.23±7.75	83.71±8.65	80.31±14.99	67.92±10.9	<0.01	0.04	<0.01
mGCC Superior Average (µm)	93.56±10.4	90.44±7.32	84.65±9.21	82.12±13.79	67.89±11.14	<0.01	0.12	<0.01
mGCC Inferior Average (µm)	95.04±9.37	90.04±8.8	82.77±10.05	78.52±17.08	67.98±13.35	<0.01	0.01	<0.01
mGCC-Focal Loss Volume (%)	1.08±1.21	1.7±2.43	2.93±3.46	6.12±3.92	9.39±4.03	<0.01	0.09	<0.01
mGCC-Global Loss Volume (%)	4.53±4.78	6.83±5.8	12.37±7.82	17.05±11.31	29.39±8.89	<0.01	0.04	<0.01
mGCC-Root Mean Square	0.07±0.05	0.08±0.04	0.09±0.05	0.14±0.05	0.17±0.06	<0.01	0.51	<0.01

Baseline clinical data of patient eye per group reported as mean ± standard deviation.
P-values from independent t-test and Jonckheere-Terpstra trend for continuous data and chi-square for categorical data
*P-values for all groups
**P-values between controls and patients with ocular hypertension
‡ P-values between patients with ocular hypertension and POAG (mild, moderate, and severe)

Table III: Vessel Ddensities of healthy controls, ocular hypertension, and glaucomatous eyes

Variables	Controls (n=69) (%)	Ocular Hypertension (n=36) (%)	Mild Glaucoma (n=54) (%)	Moderate Glaucoma (n=25) (%)	Severe Glaucoma (n=12) (%)	P value*-all groups	P value**-controls and ocular hypertension	P value ‡ -ocular hypertension and POAG
Avg SSI Disc	60.42±11.81	59.6±11.74	56.77±9.78	52.33±8.83	49±10.83	<0.01	0.74	<0.01
Whole Image ONH Vessel Density (%)	56.71±4.36	56.79±5.08	52.85±5.15	49.82±4.06	43.12±4.19	<0.01	0.94	<0.01

Inside Disc Vessel Density (%)	50.27±6.51	50.92±6.98	47.91±6.64	46.13±6.63	43.53±5.14	<0.01	0.65	<0.01
Peripapillary Vessel Density (%)	61.32±4.35	61.37±5.35	57.06±6.7	53.43±4.99	47.01±3.47	<0.01	0.96	<0.01
Nasal Vessel Density (%)	59.53±4.89	59.76±6.29	55.46±7.98	52.84±6.94	47.41±5.38	<0.01	0.84	<0.01
Inferior Nasal Vessel Density (%)	62.97±6.84	62.66±5.71	59.04±9.8	57.11±9.52	53.17±6.66	<0.01	0.82	0.01
Inferior Temporal Vessel Density (%)	66.13±4.35	64.52±8.21	60.02±9.07	54.32±10.65	45.58±9.89	<0.01	0.2	<0.01
Superior Temporal Vessel Density (%)	63.71±5.85	64.72±8.46	59.97±7.82	51.91±10.66	43.94±9.62	<0.01	0.49	<0.01
Superior Nasal Vessel Density (%)	61.29±6.02	59.16±7.91	56.56±9.43	55.14±7.05	43.45±7.74	<0.01	0.14	<0.01
Temporal Vessel Density (%)	59.29±6.62	60.64±7.3	55.56±7.68	52.22±5.22	47.21±7.3	<0.01	0.35	<0.01
Avg SSI Macular	65.83±7.49	62.79±11.7	63.13±7.74	59.01±8.28	57.24±7.47	0.01	0.18	0.18
Whole Image Macular Vessel Density (%)	50.27±4.23	49.04±4.7	48.12±3.8	46.13±3.91	43.43±4.71	<0.01	0.26	<0.01
Fovea Vessel Density (%)	27.01±5.42	26.76±5.96	27.69±5.67	24.52±6.05	27.84±8.4	0.38	0.86	0.28
Parafovea Vessel Density (%)	52.72±4.66	51.4±5.28	50.74±4.04	48.45±3.98	45.77±4.98	<0.01	0.27	<0.01
Superior-Hemi Vessel Density (%)	52.84±4.96	51.74±5.09	51.22±4.13	49±3.64	46.88±4.33	<0.01	0.37	0.01
Inferior-Hemi Vessel Density (%)	52.61±4.62	51.06±6.01	50.27±4.34	47.9±4.74	44.69±6.09	<0.01	0.22	<0.01
Temporal Vessel Density (%)	52.61±3.7	51.15±5.35	50.57±3.61	48.53±3.76	46.1±5.44	<0.01	0.17	0.01
Nasal Vessel Density (%)	51.95±5.16	50.38±6	50.74±4.36	47.07±5.28	46.25±5.01	<0.01	0.24	0.02

Baseline clinical data of patient eye per group reported as mean ± standard deviation.
P-values from independent t-test and Jonckheere-Terpstra trend for continuous data and chi-square for categorical data
*P-values for all groups
**P-values between controls and patients with ocular hypertension
‡ P-values between patients with ocular hypertension and POAG (mild, moderate, and severe)

There was no statistically significant difference among the groups in terms of BMI, family history of glaucoma, IOP, ethnicity, and self-reported history of diabetes. The groups differed by age, gender, self-reported history of hypertension, CCT, VF MD and PSD. Healthy controls had the youngest age. Patients with ocular hypertension had the highest CCT. Our patient population was ethnically diverse.

There were no significant differences in whole image ONH vessel density and macular vessel density by race (p=0.96;0.07) or gender (p=0.78;0.18).

Controls and Ocular hypertension

There was no difference in SSI for all scan types. Compared to healthy controls, patients with ocular hypertension had a significantly thicker CCT, and a smaller optic disc area, horizontal C/D, optic cup area, and cup volume.

For structural properties, patients with ocular hypertension had significantly thinner average RNFL, inferior RNFL, inferior temporal RNFL, average mGCC, inferior mGCC, and a larger GLV. We found no changes in vessel density. The most pronounced structural property change was an increase in GLV by 50.8% followed by a decrease of 8.16% in inferior temporal RNFL thickness and a decrease of 5.27% in inferior RNFL thickness.

Table IV shows the CV of whole image ONH vessel density, whole image macular vessel density, average RNFL thickness, and average mGCC thickness. Structural properties had a higher CV compared to vessel density.

All remaining variables in Table I through Table III had no significant difference between healthy controls and patients with ocular hypertension.

Table IV: Inter-patient Coefficients of Variation by group

	Control (n=69)	Ocular Hypertension (n=36)	Mild Glaucoma (n=54)	Moderate Glaucoma (n=25)	Severe Glaucoma (n=12)
Average RNFL (μm)	10.20%	10.89%	14.16%	18.78%	12.48%
mGCC Average (μm)	10.23%	8.59%	10.34%	18.66%	16.05%
Whole Image Disc Vessel Density (%)	7.70%	8.94%	9.75%	8.15%	9.72%
Whole Image Macular Vessel Density (%)	8.42%	9.59%	7.89%	8.48%	10.85%

RNFL: Retinal nerve fiber layer
mGCC: Macular ganglion cell complex

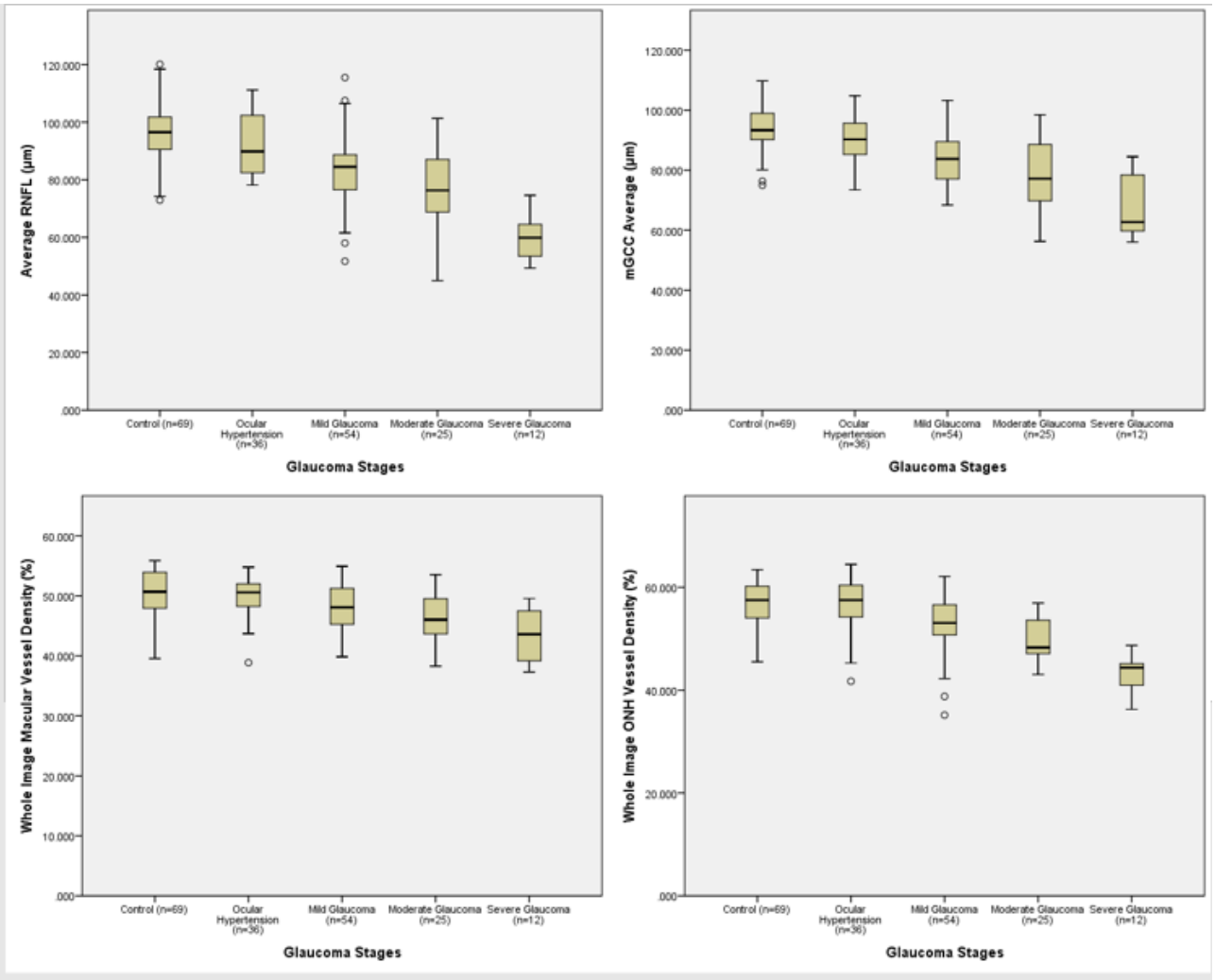
Ocular hypertension and POAG

There was a significant decrease in SSI for RNFL and ONH vessel density scans between patients with ocular hypertension and severe POAG. Whole image ONH vessel density, whole image macular vessel density, average RNFL thickness, and average mGCC thickness decreased as glaucoma progressed from ocular hypertension to severe stage as shown in Table II, Table III, and Figure 2. The most pronounced structural property or vessel density change was an increase in FLV by 68.7% followed by an increase in GLV by 50.3% and a decrease in RNFL superior temporal thickness by 7.22%.

As glaucoma progressed from ocular hypertension to severe glaucoma, the CV for vessel density measurements were smaller compared to structural property measurements. The range of CV for both vessel densities were <3%, while the range of CV for both structural properties were >7.5%.

Ethnicity, BMI, family history of glaucoma, presence of hypertension, diabetes, or cataracts, past ocular surgeries, past laser trabeculoplasties, IOP, CCT, FAZ area, optic cup area, cup volume, and fovea vessel density had no significant difference between groups.

Figure 2: Boxplots for structural properties and vessel density by patient groups



Boxplots represent average RNFL, mGCC average, whole image macular and ONH vessel density by healthy controls, patients with ocular hypertension, and with POAG (mild, moderate, and severe). The horizontal lines within the box represent the medians. The boxes represent the interquartile range between the 1st and 3rd quartile. The top whisker is the largest value within 1.5*the interquartile range from the 3rd quartile. The bottom whisker is the smallest value within 1.5*the interquartile range from the 1st quartile. Values outside these whiskers are defined as outliers and are represented by circles.
RNFL: Retinal nerve fiber layer
mGCC: Macular ganglion cell complex

Predictors of Glaucoma Progression

Table V summarizes the linear relationship of VF MD on structural properties and vessel density. Multivariate linear regression analysis showed that whole image ONH vessel density, whole image macular vessel density, average RNFL thickness, average mGCC thickness, and cataract status were predictive of worsening VF MD. The measured structural properties and vessel density were equally effective at determining the glaucoma stage.

Table V: Multivariate linear regression analyses for dependent variable, Visual Field Mean Deviation

Independent Variable	Adjusted Beta coefficient* (95% CI)	Pearson's correlation, r	p
Average RNFL (µm)	0.20 (0.16, 0.24)	0.658	<0.001

mGCC Average (μm)	0.19 (0.14, 0.24)	0.591	<0.001
Whole Image Disc Vessel Density (%)	0.68 (0.52, 0.85)	0.627	<0.001
Whole Image Macular Vessel Density (%)	0.47 (0.25, 0.70)	0.487	<0.001

*Adjusted for age, BMI, gender, ethnicity, family history of glaucoma, presence of hypertension, diabetes, or cataracts, BCVA, and SSI.
CI: confidence interval
RNFL: Retinal nerve fiber layer
mGCC: Macular ganglion cell complex

DISCUSSION

We evaluated structural properties and vessel density between healthy controls, patients with ocular hypertension, and with POAG (mild, moderate, and severe). In our patient population, we found that vessel density and structural properties assessed by OCT-A were equally effective at providing an objective measure of glaucoma damage in the eye. In addition, we found structural property decreases from healthy controls to patients with ocular hypertension but did not find vessel density decreases. This could suggest that structural damage may occur before vessel density damage in patients with ocular hypertension. These objective measures of glaucomatous damage could signify an improvement over VF tests in the diagnosis of glaucoma.

Controls and Ocular hypertension

In our patient population, we found differences in some structural properties between controls and patients with ocular hypertension, but no changes in vessel density. The most pronounced structural property change was in the RNFL inferior temporal sector. This sector corresponds to the nasal step defect seen in early visual fields abnormalities, which support that our patients with ocular hypertension have early glaucomatous damage that can be detected through RNFL changes while having normal visual fields(5,13,14).

Other studies using OCT-A have investigated vessel density changes in patients with ocular hypertension compared to healthy controls with conflicting results. Triolo et al. noticed decreases in RNFL thickness and none in ONH or macular vessel density(15). In another study, Manalastas et al. found significant RNFL thickness and ONH vessel density decreases, but none for macular vessel density(6). Finally, Yarmohammadi et al. found significant decreases for RNFL and whole image ONH vessel density, but no changes for peripapillary vessel density(16). The consistency of RNFL thickness decreases with variable vessel density decreases suggest that structural properties are affected before vessel density in early glaucomatous damage. Another possibility for the variable vessel densities between studies is that vessel density damage might be hidden by large vessels early in glaucoma. Geyman et al noted that removal of large vessels increased the accuracy of detecting mild glaucoma(8). This could suggest that increased IOP affects smaller vessels first and could account for the variable vessel density change in patients with ocular hypertension as the large vessels mask small vessel damage(8).

Ocular hypertension and POAG

In our study, we noticed a decreasing, stepwise trend in RNFL, mGCC, ONH and macular vessel density as glaucoma progressed. From the similar Pearson's coefficients, this suggests that structural properties and vessel density are equally effective at measuring glaucomatous damage. Our

study results are like those found by Geyman et al. and Chen et al. who also found that both structural properties and vessel density are similarly effective at measuring glaucoma stage(8,17).

While other studies have looked at CV in POAG using OCT-A to measure reproducibility of OCT-A(18–20), these studies have only looked at healthy controls or POAG patients overall. This study is unique because we looked at the CV between patients and across all stages of POAG. We noticed that as glaucoma progressed, the range of CV for structural properties is greater than for vessel density. This suggests that as glaucoma progresses, vessel density might have better reproducibility compared to structural properties. Therefore, for more severe cases of POAG, vessel density might provide more consistent measurements for clinical management and might be more effective for detecting changes over time(21).

LIMITATION

One limitation in this study is that our VF and OCT-A scans were not done close together. Therefore, patients could have had progression of their glaucoma and they could have a discrepancy between their VF and OCT-A scan. We attempted to mitigate this limitation by excluding any patients whose glaucoma diagnosis changed in the interim. A second limitation is that SSI has been shown to affect vessel density measurements(9). We attempted to mitigate this by including SSI in our multivariate linear regressions. A third limitation is that the Optovue RTVue XR software only supports 3mm x 3mm optic disc images for testing purposes and is not representative of their release software. This might cause some inaccuracies as images had lower resolution and the values cannot be compared to later software versions or different image sizes. A fourth limitation is that our horizontal C/D and optic cup area was greater in healthy controls than patients with ocular hypertension. This could represent a sampling bias as some of our healthy controls were referred to our eye care clinic due to concerns for glaucoma due to increased cup size. However, they were found to have no evidence of glaucomatous damage after an eye exam.

CONCLUSION

ONH and macular vessel density and structural properties assessed by OCT-A can provide an equally effective objective measure of glaucoma damage in the eye. Our study has shown that structural damage may occur before vessel density damage in ocular hypertension. For more severe POAG, vessel density might have better reproducibility. More longitudinal studies are needed to evaluate vessel density changes over the development of POAG.

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