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Original Article

Optical coherence tomography angiography analysis of the retina in patients recovered from COVID-19: a case-control study

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Objective: To quantify the density of the macular microvasculature and the area of the foveal avascular zone (FAZ) in patients recovered from coronavirus disease 2019 (COVID-19) using optical coherence tomography angiography (OCTA) analysis.

Methods: In a comparative cross-sectional, observational study, patients recovered from COVID-19 were included in this study. All included subjects exhibited a reverse transcription-polymerase chain reaction—confirmed diagnosis of COVID-19. Spectral domain macular OCTA was performed at least 2 weeks after recovery from systemic COVID-19. Vessel density (VD) of the superficial (SCP) and deep retinal capillary plexus (DCP) and the area of the FAZ were measured in COVID-19 recovered patients versus age-matched normal controls.

Results: Thirty-one recovered COVID-19 patients and 23 healthy normal controls were studied. Mean quality scan index was 7.64 ± 0.66 in the COVID cases and 8.34 ± 0.71 in the normal controls ($p = 0.001$). Mean SCP VD and DCP VD of the COVID cohort were significantly lower than the SCP VD and DCP VD of the control group in the foveal and parafoveal regions. FAZ area was greater in the COVID cohort, but this difference was not statistically significant. In addition, in the COVID cohort, VD of the SCP was lower in patients with a history of COVID-19 hospitalization versus those without such a history, but this did not reach statistical significance.

Conclusions: Patients recovered from COVID-19 displayed alterations in the retinal microvasculature, including a significantly lower VD in the SCP and DCP. Patients with coronavirus infection may be at risk of retinal vascular complications.

Objectif: Quantifier la densité de la microvasculature maculaire et l'aire de la zone fovéale avasculaire (ZFA) après une infection à coronavirus 2019 (COVID-19) grâce à l'angiographie-tomographie par cohérence optique (OCT-A, pour *optical coherence tomography angiography*).

Méthodes: Une étude d'observation comparative transversale a porté sur des patients qui ont contracté la COVID-19. Tous les sujets inclus avaient eu un diagnostic de COVID-19 confirmé par test RT-PCR (*reverse transcription-polymerase chain reaction*). Une OCT-A en domaine spectral de la macula a été réalisée au moins 2 semaines après la disparition des symptômes généraux de la COVID-19. La densité vasculaire (DV) des plexus capillaires superficiel (PCS) et profond (PCP) de la rétine, de même que l'aire de la ZFA, ont été mesurées chez les patients qui se sont remis de la COVID-19 et comparées à celles de sujets témoins en bonne santé et appariés pour l'âge.

Résultats: Ainsi, 31 patients qui se sont remis de la COVID-19 et 23 témoins en bonne santé ont été étudiés. L'indice moyen de qualité des images se chiffrait à 7,64 ± 0,66 dans le groupe COVID et à 8,34 ± 0,71 dans le groupe témoin ($p = 0,001$). La DV moyenne du PCS et du PCP dans le groupe COVID était significativement inférieure à celle du groupe témoin dans les régions fovéale et parafovéale. L'aire de la ZFA était plus grande dans le groupe COVID, mais la différence n'était pas significative sur le plan statistique. De même, la DV du PCS et du PCP des patients du groupe COVID était moindre chez ceux qui ont dû être hospitalisés en raison de la COVID-19 que chez ceux qui n'ont pas été hospitalisés, bien que la différence n'ait pas atteint le seuil de signification statistique.

Conclusion: Les patients qui se sont rétablis après un diagnostic de COVID-19 ont présenté des anomalies de la microvasculature rétinienne, dont une DV significativement réduite dans le PCS et le PCP. Les patients qui sont infectés par le coronavirus sont donc exposés à un risque de complications vasculaires rétiniennes.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly pathogenic human coronavirus, which can cause serious life-threatening respiratory illness, namely, severe pneumonia,[1] and even multiorgan failure.[2,3] Despite the growing body of knowledge about various clinical presentations and fatal consequences of coronavirus disease 2019 (COVID-19), reports regarding ocular manifestations are uncommon.[4,5]

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Virus replication starts after binding to epithelial cells in the upper respiratory tract with subsequent propagation and migration down the respiratory tract triggering the innate immune response. Angiotensin-converting enzyme (ACE) 2 has been identified as the main receptor for SARS-CoV2[6] and its receptors are present in cell membranes of type II alveolar cells in the lung and enterocytes of the small intestine, and also in the arterial and venous endothelial cells and arterial smooth muscle cells of most organs.[7,8] ACE and ACE2 have been found in the choroid and in different cell types of the retina, including Müller cells, ganglion cells, retinal vascular endothelial cells, and photoreceptor cells.[9]

Reports on the ocular manifestations of COVID-19 mostly describe anterior segment disorders, including conjunctival congestion, chemosis, and conjunctivitis.[10,11] Reports of the retinal findings are rare. One group from Brazil[12] described various retinal complications of COVID-19, but the validity of this study has been called into question.[13]

Optical coherence tomography angiography (OCTA) can provide depth-resolved imaging of blood flow in the retina and choroid with microvascular detail that exceeds the capability of other forms of imaging.[14] This study evaluated patients previously infected with coronavirus, using OCTA analysis to assess the retinal microvasculature. The objective of this study was to measure the vessel density (VD) of the retinal capillary plexuses and the area of the foveal avascular zone versus an age-matched normal control group.

Materials and Methods

Study Participants

A cross-sectional study was conducted at the Imam Reza Hospital, Mashhad University of Medical Sciences (MUMS), Mashhad, Iran. Patients with a definite history of COVID-19, confirmed by a positive test result with real-time, reverse transcription-polymerase chain reaction of a nasopharyngeal swab sample, and with a history of recovery from the systemic symptoms for at least 2 weeks, were included. Detailed ocular and systemic histories were obtained from each subject. Patients recruited for this study were all doctors and nurses from the Imam Reza Hospital who had recovered from COVID-19 and all volunteered to undergo ophthalmological examination and OCTA analysis for the purpose of this research investigation.

Exclusion criteria included any history of refractive or intraocular surgery. Any patients who admitted to a history of diabetes mellitus, auto-immune disease, current pregnancy, breastfeeding, or migraine were also excluded. Additional exclusion criteria included absolute spherical refractive error greater than 5 diopters and cylindrical refractive error more than 2 diopters. Patients with evidence of glaucoma or clinically apparent retinal disease were also not enrolled. Any evidence of ocular media opacity preventing high-quality imaging or reduced OCTA scan quality (i.e., quality scan index less than 7/10) were also excluded from the analysis. Any subjects with best-corrected visual acuity less than 20/20 were also not included in the protocol.

The age-matched control cohort comprised normal individuals: nurses and physicians from the MUMS who were imaged on the same OCTA machine at the Imam Reza Hospital in 2019 as part of a prior study to build a local OCTA normative database.

Complete history regarding the patients' symptoms, disease course, and hospitalization were recorded. Oxygen saturation at the time of examination was measured by a portable pulse oximeter (Nonin 7500 Pulse Oximeter, Nonin Medical Inc, Plymouth, Minn) and refraction was evaluated using a KR-1 Auto Kerato-Refractometer (Topcon Medical Systems, Inc, Tokyo, Japan).

Image Acquisition and Analysis

All OCTA scans were performed with the AngioVue (RTVue XR Avanti, Optovue, Fremont, Calif; Software Version 2018.0.0.14) system with an A scan rate of 70,000 scans per second, a light source of 840 nm, and a band width of 45 nm. Each B scan was repeated for image decorrelation and macular cubes were performed in the horizontal and vertical orthogonal directions. All measurements were acquired using the automated default segmentation with the preset settings for the superficial retinal capillary plexus (SCP) and the deep retinal capillary plexus (DCP). The AngioRetina 3 × 3 mm scan (304 lines × 304 A-scans) protocol with AngioVue 3D Projection Artifact Removal was used.

All images were centred on the fovea and displayed a quality scan index of at least 7/10. All images with quality scan index less than 7 were excluded. All images in the study were carefully reviewed by the first author (M.A.) and the senior authors (V.S., D.S.) to ensure sufficient quality and resolution, and any images with motion artefact significant enough to interfere with vessel density analysis as determined by both the first author (M.A.) and senior authors (V.S., D.S.) were also excluded.

For the 3 × 3 mm scans, measurements of the fovea avascular zone (FAZ, including FAZ area, the perimeter circumference of the FAZ [PERIM], and foveal vessel density [FD]) and of the vascular density (VD) of the fovea and parafovea at the level of the SCP and DCP were recorded from the AngioAnalytic report. Parafovea was defined as a ring around the fovea with diameter of 3 mm. All the images were checked for segmentation errors by 2 retina specialists (M.A. and M.M.).

Statistical Analysis

The normal distribution of variables was examined using the Shapiro-Wilk test, and normality plots and homogeneity of variances were ascertained by Levene's test. Based on data distribution and type, the independent-samples *t* test, paired *t* tests, Mann-Whitney *U* test, or Pearson correlation test were used for comparisons. For categorical variables, χ^2 test and Fisher's exact test were used. Multivariate linear regression analysis and multivariate logistic regression analysis were

performed to address the effect of potential confounders on continuous and nominal dependent variables, respectively. For variables without normal distribution, the variable was logarithm transformed and then entered into the regression model. Control of concomitant continuous independent variables (covariates) was performed using multivariate analysis of covariance (MANCOVA). After performing MANCOVA, revised *p* values were added to tables and multivariate analysis results were reported. The level of statistical significance was set at 0.05. All statistical analyses were performed using the SPSS program for Windows, version 20 (IBM SPSS Statistics, IBM Corporation, Chicago, Ill).

Ethical Considerations

The study protocol adhered to the tenets of the Declaration of Helsinki. All participants provided written informed consent before enrollment, and the ethical aspects of the study were approved by the Regional Committee on Medical Ethics at Mashhad University of Medical Sciences, Mashhad, Iran (IR.MUMS.REC.1399.104).

Results

Thirty-one recovered COVID-19 patients (14 females, 45.2%) with a mean age of 40.4 ± 9.2 years and 23 healthy normal controls (9 females, 39.1%) with a mean age of 36.6 ± 7.1 years were enrolled in the study after exclusion criteria were applied. Age ($p = 0.115$) and sex ($p = 0.658$) were not significantly different between the 2 groups.

In total, 3 cases were excluded from the analysis. Two patients were excluded in the COVID group because the quality scan score was less than 7 and because scans displayed significant motion artefact. An additional case was excluded from the normal cohort to a quality scan score less than 7.

All patients were symptom-free for at least 2 weeks. Medical history was otherwise unremarkable for all of the patients and controls, except for 2. None of the COVID patients or controls endorsed a history of diabetes mellitus, and only 2 cases in the COVID group disclosed a history of hypertension that was well controlled with medications or diet. Except for these 2 subjects, the other patients did not provide any history of chronic drug consumption.

For all subjects, only the data of the eye with better image quality were used for analysis. Nine patients (29%) endorsed a history of hospitalization for COVID-19. O_2 saturation was in the normal range (94%–99%) in these patients and was not different between the hospitalized and nonhospitalized patients ($p = 0.616$). None of the hospitalized patients required invasive ventilation. None of the patients received steroid as these agents were not yet introduced into the COVID-19 treatment protocol. Face mask oxygen supplementation was used in 6 of 9 hospitalized patients. Although visual acuity was 20/20 in all COVID cases and normal controls at examination, 2 of the hospitalized patients (22.2%) and 4 of the nonhospitalized patients (18.1%) admitted to a history of blurred vision during their symptomatic period that subsequently resolved.

Mean quality scan index was 7.64 ± 0.66 in the COVID cases and 8.34 ± 0.71 in the normal controls ($p = 0.001$). None of the images included in the final analysis displayed segmentation error.

The 3 × 3 mm mean whole-image SCP VD in the COVID-19 group (44.98 ± 4.16) was significantly lower than the mean SCP VD in the normal control group (48.36 ± 2.24) ($p = 0.001$) (Table 1). The 3 × 3 mm mean whole-

Table 1—Vascular density of the superficial and deep retinal capillary plexuses in the foveal and parafoveal regions in recovered patients with COVID-19 versus normal controls

	Normal Eyes, Mean ± SD (Range)	COVID-19 Patients' Eyes, Mean ± SD (Range)	*P* Value* (Compared with Normal)	*P* Value* (Compared with Normal) with Multivariate Analysis
Whole-image SCP VD	48.36 ± 2.24 (42.70–52.60)	44.98 ± 4.16 (29.30–51.80)	**0.001**	0.073
Superior-hemi SCP VD	48.32 ± 2.28 (43.30–52.40)	44.85 ± 4.12 (30.20–52.30)	**0.001**	**0.048**
Inferior-hemi SCP VD	48.41 ± 2.39 (42–52.90)	45.10 ± 4.26 (28.40–51.30)	**0.002**	0.101
Fovea SCP VD	21.10 ± 5.35 (12.70–31.40)	16.70 ± 5.35 (4.20–29.20)	**0.004**	**0.003**
Parafovea SCP VD	51.23 ± 2.56 (45.80–55.60)	47.91 ± 4.46 (31.70–55.40)	**0.002**	0.160
Parafoveal superior-hemi SCP VD	51.18 ± 2.63 (45.80–55.60)	47.86 ± 4.43 (32.80–55.70)	**0.002**	0.154
Parafoveal inferior-hemi SCP VD	51.30 ± 2.75 (44.90–55.70)	47.98 ± 4.62 (30.50–55.40)	**0.004**	0.188
Parafoveal temporal SCP VD	49.38 ± 2.35 (43.90–54)	46.68 ± 4.49 (29.70–54)	**0.011**	0.271
Parafoveal superior SCP VD	52.19 ± 3.32 (45.80–57.70)	48.39 ± 4.62 (33.40–56.30)	**0.001**	0.099
Parafoveal nasal SCP VD	50.14 ± 3.05 (42–55.30)	47.47 ± 4.81 (31.70–55.60)	**0.024**	0.755
Parafoveal inferior SCP VD	53.36 ± 2.83 (46.50–57)	49.13 ± 4.60 (31.90–56.40)	**<0.001**	**0.027**
Whole-image DCP VD	53.03 ± 3.29 (43.50–57.50)	49.74 ± 3.39 (43.40–55.70)	**0.001**	**0.044**
Superior-hemi DCP VD	53.48 ± 3.47 (43.70–58.20)	49.62 ± 3.39 (42.80–54.90)	**<0.001**	**0.011**
Inferior-hemi DCP VD	52.54 ± 3.25 (43.20–57.20)	49.85 ± 3.53 (43.90–56.40)	**0.006**	0.164
Fovea DCP VD	37.61 ± 4.92 (29.40–48.60)	32.63 ± 6.50 (14.10–45.40)	**0.003**	**0.002**
Parafoveal DCP VD	54.62 ± 3.33 (45.20–58.80)	52.12 ± 3.53 (45.80–58.50)	**0.011**	0.263
Parafoveal superior-hemi DCP VD	55.11 ± 3.47 (45.20–59.80)	52.06 ± 3.51 (46.30–58.20)	**0.003**	0.089
Parafoveal inferior-hemi DCP VD	54.12 ± 3.33 45.30–59.70)	52.19 ± 3.68 (45.20–58.80)	0.053	0.642
Parafoveal temporal DCP VD	54.06 ± 3.50 (43.30–58.70)	52.49 ± 3.35 (45.30–57.80)	0.103	0.789
Parafoveal superior DCP VD	55.04 ± 3.67 (44.90–59.50)	51.42 ± 4.09 (43.90–58.60)	**0.002**	0.062
Parafoveal nasal DCP VD	55.15 ± 3.29 (46.90–59.60)	52.72 ± 3.57 (46.50–59.80)	**0.014**	0.270
Parafoveal inferior DCP VD	54.20 ± 3.38 (45.90–60.90)	51.84 ± 3.99 (44.40–59.20)	**0.026**	0.379

SCP, superficial retinal capillary plexus; DCP, deep retinal capillary plexus; VD, vascular density; NA, not available.
* Statistically significant *P* values are in Bold.

Table 2—Measurements of the FAZ (including FAZ area, PERIM, and FD) in recovered patients with COVID-19 versus normal controls

	Normal Eyes, Mean ± SD (Range)	COVID-19 Patients' Eyes, Mean ± SD (Range)	*P* Value (Compared with Normal	*P* Value* (Compared with Normal) with Multivariate Analysis
FAZ	0.24 ± 0.08 (0.07–0.35)	0.27 ± 0.11 (0.07–0.57)	0.191	**0.025**
PERIM	1.91 ± 0.37 (1.14–2.42)	2.07 ± 0.40 (1.30–3.01)	0.126	**0.016**
FD	51.59 ± 3.38 (40.49–55.08)	50.23 ± 4 (40.65–56.60)	0.197	0.556

FAZ, fovea avascular zone; PERIM, perimeter circumference of the FAZ; FD, foveal vessel density.
* Statistically significant *P* values are in Bold.

Table 3—Vascular density of the superficial and deep retinal capillary plexuses in the foveal and parafoveal regions in hospitalized and nonhospitalized patients with COVID-19

	Nonhospitalized, Mean ± SD (Range), N = 22	Hospitalized, Mean ± SD (Range), N = 9	*P* value
Whole-image SCP VD	45.177 ± 4.35 (29.30–51.80)	44.51 ± 3.85 (38.30–51.50)	0.693
Superior-hemi SCP VD	45 ± 4.24 (30.20–52.30)	44.50 ± 4.05 (38.10–52.30)	0.763
Inferior-hemi SCP VD	45.34 ± 4.55 (28.40–51.30)	44.50 ± 3.65 (38.50–50.60)	0.625
Fovea SCP VD	16.09 ± 4.54 (6.10–25.30)	18.22 ± 7.05 (4.20–29.20)	0.323
Parafovea SCP VD	48.13 ± 4.67 (31.70–55.40)	47.40 ± 4.13 (40.70–55)	0.686
Parafoveal superior-hemi SCP VD	47.98 ± 4.55 (32.80–55.40)	47.56 ± 4.35 (41–55.70)	0.817
Parafoveal inferior-hemi SCP VD	48.29 ± 4.91 (30.50–55.40)	47.23 ± 3.96 (40.40–54.20)	0.572
Parafoveal temporal SCP VD	46.77 ± 4.74 (29.70–53.60)	46.45 ± 4.08 (40.30–54)	0.860
Parafoveal superior SCP VD	48.58 ± 4.83 (33.40–56.30)	47.92 ± 4.28 (41.90–55.30)	0.725
Parafoveal nasal SCP VD	47.64 ± 4.89 (31.70–55.20)	47.05 ± 4.86 (38.5–55.60)	0.764
Parafoveal inferior SCP VD	49.54 ± 4.86 (31.90–56.40)	48.12 ± 3.95 (41.80–54.90)	0.444
Whole-image DCP VD	49.70 ± 3.60 (43.40–55.30)	49.85 ± 3.03 (45.70–55.70)	0.910
Superior-hemi DCP VD	49.59 ± 3.65 (42.80–54.90)	49.71 ± 2.86 (46.20–54.90)	0.930
Inferior-hemi DCP VD	49.79 ± 3.69 (43.90–55.60)	50.29 ± 3.30 (45.20–56.40)	0.884
Fovea DCP VD	32.07 ± 5.11 (18.10–40.20)	34.10 ± 9.32 (14.10–45.40)	0.463
Parafoveal DCP VD	52.16 ± 3.61 (45.80–58.50)	52.02 ± 3.54 (46.60–58)	0.919
Parafoveal superior-hemi DCP VD	52.16 ± 3.64 (46.30–58.20)	51.82 ± 3.38 (46.70–57.50)	0.809
Parafoveal inferior-hemi DCP VD	52.18 ± 3.74 (45.20–58.80)	52.21 ± 3.75 (46.50–58.50)	0.987
Parafoveal temporal DCP VD	52.41 ± 3.54 (45.30–57.80)	52.68 ± 3.02 (48.80–56.80)	0.843
Parafoveal superior DCP VD	51.53 ± 4.19 (43.90–58.20)	51.16 ± 4.06 (45.30–58.60)	0.824
Parafoveal nasal DCP VD	52.93 ± 3.81 (46.50–59.80)	52.22 ± 3.08 (46.70–57.40)	0.622
Parafoveal inferior DCP VD	51.79 ± 3.91 (44.40–58.30)	51.97 ± 4.43 (45.60–59.20)	0.911

SCP, superficial retinal capillary plexus; DCP, deep retinal capillary plexus; VD, vascular density.

image DCP VD in the COVID-19 cohort (49.74 ± 3.39) was also significantly lower than the mean DCP VD of the control cohort (53.03 ± 3.29) ($p = 0.001$) (Table 1).

The mean FAZ area in the COVID cohort (0.27 ± 0.11) was not significantly greater ($p = 0.191$) than the mean FAZ area in the control cohort (0.24 ± 0.08) (Table 2).

We applied a one-sample MANCOVA to identify the effects of scan quality on SCP and DCP VD comparisons between cases and controls, and considered the quality scan index a covariate of interest. After MANCOVA analysis, statistical significance in the comparison of the various SCP and DCP VD datasets versus normal controls was maintained in several VD comparisons, whereas others lost statistical significance. Of note, fovea SCP and DCP VD and superior hemisphere SCP and DCP VD and whole-image DCP VD all maintained statistically significant lower VD values versus controls after MANCOVA. Interestingly, FAZ area and FAZ PERIM (perimeter circumference of the FAZ) were significantly greater after MANCOVA versus normal controls (p values of 0.025 and 0.016, respectively).

Of note, mean SCP VD and DCP VD and FAZ area were not statistically significantly different in the hospitalized group compared with the nonhospitalized COVID-19 group (Tables 3 and 4) although the mean SCP VD was consistently lower in the hospitalized patients.

All scans were qualitatively evaluated for evidence of frank microvascular abnormalities, and 4 COVID cases did demonstrate apparent flow deficit, especially in the SCP. No frank microvascular abnormalities such as microaneuysms or beading were detected (Fig. 1). Note that structural OCT was evaluated in all cases and failed to display any abnormalities.

Table 4—Measurements of the FAZ (including FAZ area, PERIM, and FD) in hospitalized and nonhospitalized patients with COVID-19

	Nonhospitalized, Mean ± SD (Range), N = 22	Hospitalized, Mean ± SD (Range), N = 9	*P* value
FAZ	0.29 ± 0.08 (0.17–0.53)	0.25 ± 0.16 (0.07–0.57)	0.394
PERIM	2.13 ± 0.30 (1.70–2.84)	1.93 ± 0.58 (1.30–3.01)	0.343
FD	50.89 ± 3.05 (44.20–56.60)	48.62 ± 5.62 (40.65–56.50)	0.279

FAZ, fovea avascular zone; PERIM, perimeter circumference of the FAZ; FD, foveal vessel density.

Discussion

In the present study, OCTA was performed to compare the VD of the retinal capillary microvasculature in a relatively young cohort of recovered COVID-19 patients versus age-matched normal controls. The course of COVID-19 was relatively mild, with a minority of patients requiring

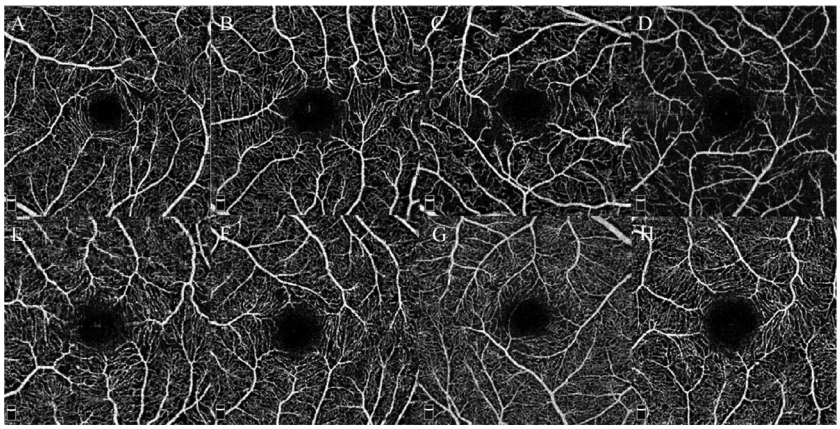

Fig. 1—En-face optical coherence tomography angiograms segmented at the level of the superficial retinal capillary plexus from 4 recovered COVID-19 patients (A–D) versus 4 age-matched normal controls (E–H). Note the remarkable flow deficits present in the en-face angiograms from the COVID cases.

hospitalization. Mean macular SCP VD and DCP VD were significantly reduced in the COVID cohort versus the age-matched controls. FAZ area was also greater in the COVID group, but this did not reach statistical significance. Qualitative analysis identified apparent flow deficits in 4 cases although frank microvascular abnormalities were not detected. This analysis did not detect any significant differences in SCP and DCP VD and FAZ area in the hospitalized versus the nonhospitalized cohorts.

In a recent case series, Marinho and colleagues reported cotton-wool spots and microhemorrhages in 4 patients suggestive of an inner retinal ischemic process.[12] The authors also reported abnormalities with OCT imaging, but a subsequent editorial letter indicated that these findings represented normal vascular landmarks.[13] Savastano et al identified reduced perfusion density of the radial peripapillary capillary plexus in recovered COVID-19 patients versus age-matched controls using OCTA analysis.[15] A systematic analysis comparing retinal capillary abnormalities in COVID patients versus age-matched normal controls has not yet been performed to our knowledge.

In an autopsy study, Casagrande and associates detected SARS-CoV-2 viral ribonucleic acid in the retina of patients who had died owing to COVID-19.[16] Given the presence of ACE2 receptors in various layers of the retina and choroid, pathoanatomical abnormalities in these ocular tissues may be expected. Reports of microvascular injury and thrombosis in patients with severe COVID-19 infection would appear to highlight the importance of evaluating retinal vascular involvement with this disease.[17]

In this study, the SCP VD and DCP VD were significantly reduced versus age-matched controls. Although the FAZ area and circumference were also numerically increased in these comparative analyses, the differences were not statistically significant. This may be because the study was underpowered to detect small differences. OCTA analysis can be an invaluable tool in the detection of retinal vascular disease in systemic disorders like diabetes before the onset of clinically evident retinopathy. This technology may detect microvascular abnormalities such as microaneurysms, venous beading, enlargement of the FAZ, and capillary nonperfusion in diabetic eyes without any clinical signs of retinopathy.[17–19] OCTA microvascular abnormalities and quantitative flow deficit analysis have been closely correlated with the clinical stage of retinopathy, and therefore OCTA can provide a biomarker of retinal disease in patients with systemic disease such as diabetes.[20] OCTA may provide similar benefits in patients with other systemic disorders such as COVID-19 as the VD reductions in this study are comparable to those in patients with diabetes.

The causes of the retinal capillary alterations detected in this study are unclear. Although direct coronavirus infection of the retina is possible, secondary effects of inflammation cannot be excluded. Exacerbation of underlying systemic diseases is unlikely given the young age of the cohort analyzed and the absence of pre-existing systemic disorders, although 2 cases did endorse a history of medically controlled hypertension. Although our cohort of 31 cases with inactive disease may not be representative of the much larger population of infected COVID patients worldwide, it is interesting to note that retinal capillary alterations were detected in our study even though the affected cohort was young without pre-existing systemic illness, indicating that potentially more serious retinal complications may develop in higher-risk COVID subjects.

This study has limitations. First, ascertainment bias may have been a factor during recruitment given that patients with more severe disease may be more likely to volunteer for the study. However, as previously mentioned, all patients in this analysis had fully recovered from their COVID infection and presented with 20/20 vision and no symptoms of vision loss. Second, our study included a relatively small sample size of over 30 patients and could be improved by a larger-scale OCTA analysis performed during the symptomatic phase of the disease. Longitudinal testing with repeat imaging at fixed intervals could provide valuable information regarding both the short- and long-term effects of COVID-19 on the retinal vascular system. Third, although this study strictly applied a quality scan threshold of greater

than 7 for every case included in the final analysis, there was still a slightly greater scan quality in the normal controls versus the affected COVID cases. The quality of the scan can be a factor affecting the vessel density analysis.[21,22] Although the ocular media was clear in all patients tested, ocular surface irregularities can degrade the quality scan index and dry eye has been associated with COVID infection,[23,24] although none of the patients in this study complained of this problem. Given the overall high-quality score greater than 7 in the COVID cases and the margin of difference versus the controls of less than 1, the significant differences in VD between these 2 groups are likely owing to pathoanatomical factors. Moreover, after multivariable analysis using MANCOVA to control for the quality scan index difference, statistical significance was maintained with some key parameters, but not with all comparisons, indicating that although the quality of the scans can influence vessel density outcomes, the lower VD values in the COVID patients are likely real. Moreover, multivariable analysis indicated that the FAZ area and FAZ perimeter circumference were significantly greater in the COVID cohort. However, further validation of our findings will be essential to ensure that the microvascular reductions in COVID recovered eyes identified in this study are the result of true anatomical alterations and not the result of artefact such as a reduced quality scan or signal strength index. Finally, although we demonstrated statistically significant microvascular differences, the clinical relevance of this finding is unclear, as the patients were all asymptomatic with 20/20 vision at the time of this analysis, although some did note vision disturbances during the active infection. It is unclear if the findings in this specific subset of COVID recovered patients may be representative of the larger population of COVID patients worldwide. Our cohort of nurses and physicians may represent a nonrandom sample of the population. Nevertheless, the OCTA analysis of 31 patients with reverse transcription-polymerase chain reaction-confirmed COVID-19 positivity and the comparison with an age-matched normal control group, similarly imaged, is novel and may highlight the importance of continued vigilance for the detection of ocular and retinal complications secondary to COVID-19 as the pandemic evolves.

In conclusion, our study demonstrated significant retinal vascular alterations in patients with a history of COVID-19, including reduced vessel density in the SCP and DCP in the foveal and parafoveal regions. The potential involvement of the retina by COVID-19 warrants a further larger-scale study that may be more representative of the ever-growing population of patients infected with COVID-19 worldwide.

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