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ORIGINAL RESEARCH

Evaluation of Retinal Vascularity Index in Patients with COVID-19: A Case–Control Study

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ABSTRACT

Introduction: The purpose of this study was to evaluate the impact of COVID-19 infection on retinal microvasculature by topographically mapping the retinal arteriole-to-venule ratio (AVR).

Methods: In a comparative cross-sectional case–control study, fundus photos were obtained in COVID-19-infected patients and healthy controls. AVT was measured over 16 points across the retina using retinal vascularity index (RVI)—a novel semi-automated computerized parameter based on retinal vasculature.

Results: A total of 51 COVID-19-positive patients and 65 healthy controls were enrolled in the study. Overall, the mean RVI of all 16 points across the retina was 0.34 ± 0.02 in patients with COVID-19 and 0.33 ± 0.02 in control subjects ($p = 0.64$). Out of the 16 points being measured, three points had a statistically significant greater value in patients with COVID compared to normal controls.

Conclusion: Localised greater RVI values were found in some of the points in COVID-19-positive patients, which likely indicates a more focal change of the vasculature.

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Key Summary Points

Why carry out this study?

SARS-CoV-2 may target vascular pericytes in the eye expressing angiotensin-converting enzyme 2, and the eye may serve as a portal of entry and reservoir for viral transmission.

Infection within the eye could potentially lead to complement-mediated endothelial cell dysfunction, microvascular damage and ocular circulation involvement.

Our study explored the effect of COVID-19 infection on the retinal microvasculature.

What was learned from this study?

Patients with COVID-19 had significantly greater retinal vascularity index values compared to non-infected patients at focal points of the retina, representing focal microangiopathy.

Possible pathomechanisms for the focal microangiopathy within the retina include local retinal pericyte dropout or a virus-induced local hypercoagulable state.

INTRODUCTION

On 30 January 2020, the World Health Organization (WHO) declared the coronavirus outbreak a global public health emergency. Since China reported its first cases to the WHO in December 2019, authorities in 219 countries and territories have reported about 143.9 million COVID-19 cases and 3.1 million deaths. The novel virus, referred to as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is an enveloped, positive-sense, single-stranded RNA virus belonging to the Betacoronavirus genus of the *Coronaviridae* family that has been isolated from a broad range of vertebrates, including humans [1].

SARS-CoV-2 RNA has been found in the tears of infected patients, and reports suggest that the ocular surface could serve as a portal of entry and a reservoir for viral transmission [2]. Various direct or indirect ocular manifestations have also emerged, including viral conjunctivitis, retinal changes like microhaemorrhages, cotton wool spots and subtle findings like hyperreflective lesions in the inner layers on optical coherence tomography (OCT) [3]. Microangiopathy of the retina is worthy of interest because it is a predisposing factor for thrombosis of small vessels, which may eventually result in organ ischaemia [4]. Since COVID-19 is able to target vascular pericytes expressing angiotensin-converting enzyme 2 (ACE-2), viral infection could potentially lead to complement-mediated endothelial cell dysfunction, microvascular damage and thus ocular circulation involvement [5].

Therefore, studying retinal vascular alteration may unveil possible COVID-19 implications in retinal vascular pathology and potentially correlate with other systemic vascular changes. Invernizzi et al. reported increased mean diameters of both retinal veins and arteries in COVID-positive patients in their study [6]. However, of note that study did not measure the retinal arteriole-to-venule ratio (AVR), which is a parameter of particular importance. AVR is a retinal vascular biomarker related to the risk of developing various systemic diseases, including diabetes, cardiovascular disease and cerebrovascular complications [7–9]. Estimating AVR is a simple but useful clinical technique in routine ophthalmic practice. Over the years, extensive efforts have been made to improve its shortcomings and revolutionize AVR estimation though the development of new techniques and parameters.

In this study, we evaluated the impact of COVID-19 infection on the retinal microvascular structure by assessing the global and focal retinal AVR across the retina, a parameter not assessed in previous studies, and comparing this between healthy subjects and subjects with acute COVID-19 infection. Furthermore, we

also assessed the retinal microvasculature using a newly developed method of evaluating AVR called the retinal vascularity index (RVI), which is a novel retinal vascular parameter measured using semi-automated retinal imaging processing software.

METHODS

This was a comparative, cross-sectional, case–control study conducted at Masina Hospital and Aditya Jyot Eye Hospital located in Mumbai, India. This study adhered to the tenets of the Declaration of Helsinki and was approved by the local ethics committee of the two centres. Written informed consent was obtained from all participants. Patients with acute COVID-19 infection, confirmed by a positive test result with real-time, reverse transcription-polymerase chain reaction (RT-PCR) of a nasopharyngeal swab sample, were recruited in the study. A group of asymptomatic subjects with no current or previous COVID-19 infection were enrolled as controls.

The inclusion criteria included RT-PCR-positive patients for SARS-CoV-2 aged between 21 and 90 years. Patients were enrolled irrespective of the presence, absence or severity of clinical signs and symptoms. The exclusion criteria included patients with known pre-existing retinal disease as identified on past medical records, patients less than 21 years or more than 90 years of age, patients who were critically ill or were not ambulatory and patients who had received any form of COVID-19 vaccination.

Image Acquisition and Analysis

All enrolled study subjects underwent pupillary dilation for both eyes using mydriatic drops (tropicamide 1%) prior to the acquisition of retinal images. Fundus photos, one for each eye, were acquired in all subjects with the Forus Health's 3Nethra Classic (India) fundus camera. Two retinal images of each eye were obtained, one centred on the optic disc and another centred on the fovea [Early Treatment of Diabetic Retinopathy Study (ETDRS) standard fields 1 and 2]. Photos with poor quality due to artefact and media opacities were not included in the analysis. If both eyes were graded "unreadable", the subject was excluded.

The retinal images were processed using an innovative purpose-built semi-automated software allowing recognition of the disc centre (https://ocularimaging.net/project-rvi/hihg9/show). This software was specifically built for analysing arteriolar and venular calibers for colour fundus images, irrespective of whether the images were disc centred or macula centred. Furthermore, the software does not rely on high image resolution and is able to compute artery and venous calibers even for lower resolution images.

The software automatically divided the photo into four quadrants and eight zones (superotemporal, ST1 & ST2; superonasal, SN1 & SN2; inferotemporal, IT1 & IT2; inferonasal, IN1 & IN2) centred at the disc (Fig. 1). Two largest arterioles and venules coursing through each zone were selected by the grader to analyze their calibers at four consecutive points at a distance between 0.5 and 1 disc diameters (DD) from the optic disc margin (Fig. 2). The selected area (0.5–1 DD from disc margin) is the most commonly reported area of interest in retinal vascular diameter assessment in the majority of studies [10]. A trained grader, masked to COVID-19 status of the group, performed the vessel measurements on the optic disc-centred image of both eyes using semi-automated software. The software allows the grader to mark the vessel of interest and label it as artery or vein (Fig. 2). Then the software automatically recognised the points being marked including their location and mean area was first calculated (= sum of [$\pi/4 \times \text{diameter}^2$] for all measurements/count [measurements] around a focal point). Subsequently, retinal vascularity index (RVI) represented by AVR (= mean area of arteriole in the particular point/mean area of vein) in the particular point was then generated for each point and extracted. RVI value was subsequently calculated at each point with a total four values in each zone and 16 values for one eye.

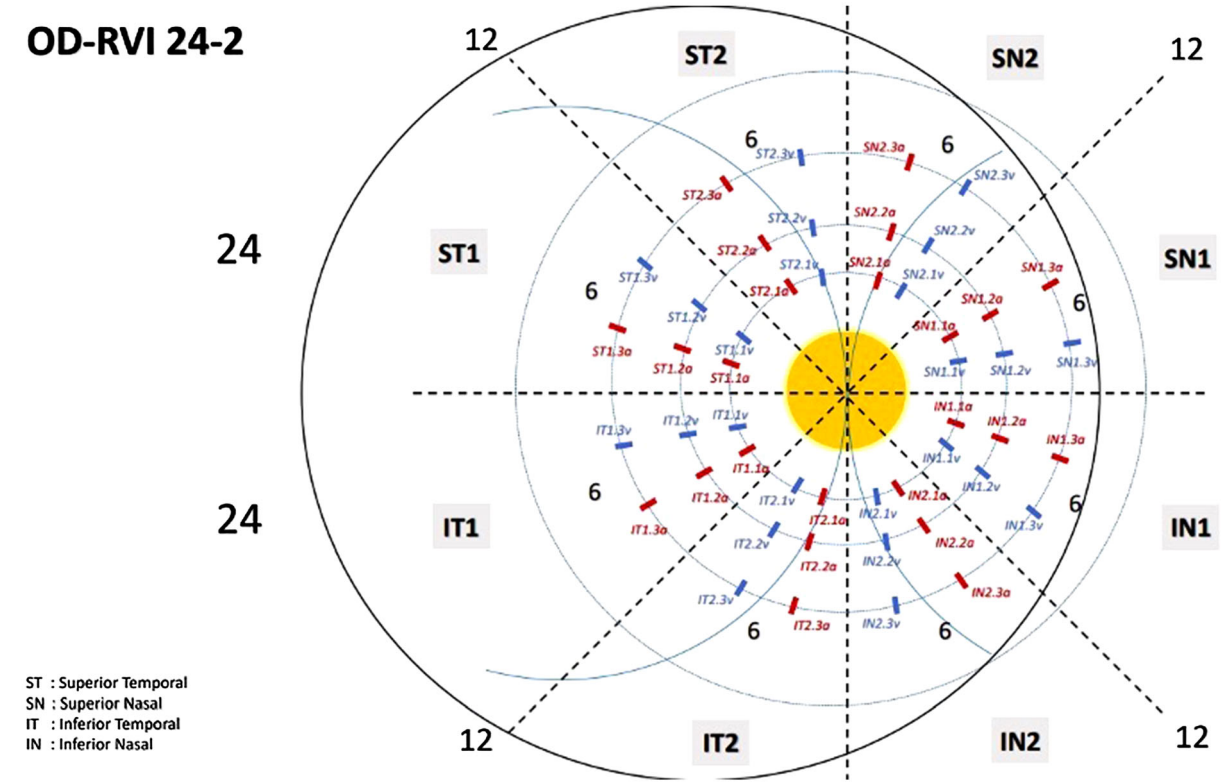

Fig. 1 Diagrammatic representation of retinal vascularity index (RVI) topographical map with disc as the centre and presence of concentric circles at 0.5 disc diameter (DD), 1.0 DD, 1.5 DD and 2.0 DD from the disc. The software is trained to automatically divide the photo into four quadrants and eight zones (superotemporal, ST1 & ST2; superonasal, SN1 & SN2; inferotemporal, IT1 & IT2; inferonasal, IN1 & IN2) centred at the disc. At each of the zones, the retinal vascular index would be calculated by using the ratio of area of retinal arteries and venules and hence giving us the arteriole-to-venule ratio at various focal points in superior and inferior halves of the retina

Statistical Analysis

Statistical analyses were performed using SPSS (IBM SPSS Statistics, Version 27, IBM Corp, New York, USA) with statistical significance (p value) evaluated at 5%. The demographic characteristics (i.e. age and gender) and RVI parameters were compared between healthy (control) and diseased (cohort) subjects. All numerical variables were expressed as mean [standard deviation (SD) or 95% confidence interval (CI 95%)]. Categorical variables were described using frequency (N) and percentage (%). Comparisons of demographics (numerical and categorical variables) between groups were performed using unpaired t test or Pearson chi-square test, respectively. RVI parameters were analysed using a linear mixed model to account for the use of both eyes (right and left eye), while treating age, gender, pre-existing diabetes melliltus and pre-existing hypertension as covariates. Further subgroup analysis of study subjects was done on the basis of the duration of disease (since the onset of COVID-19 symptoms). The observation for both right and left eye of each patient was treated as a repeated observation, with repeated covariance type set as diagonal.

RESULTS

A total of 116 subjects (221 eyes) were enrolled in the study. There were 65 healthy normal controls (36 men, 55.4% vs. 29 women, 44.6%) with a mean age of 41.7 ± 10.7 years and 51 in

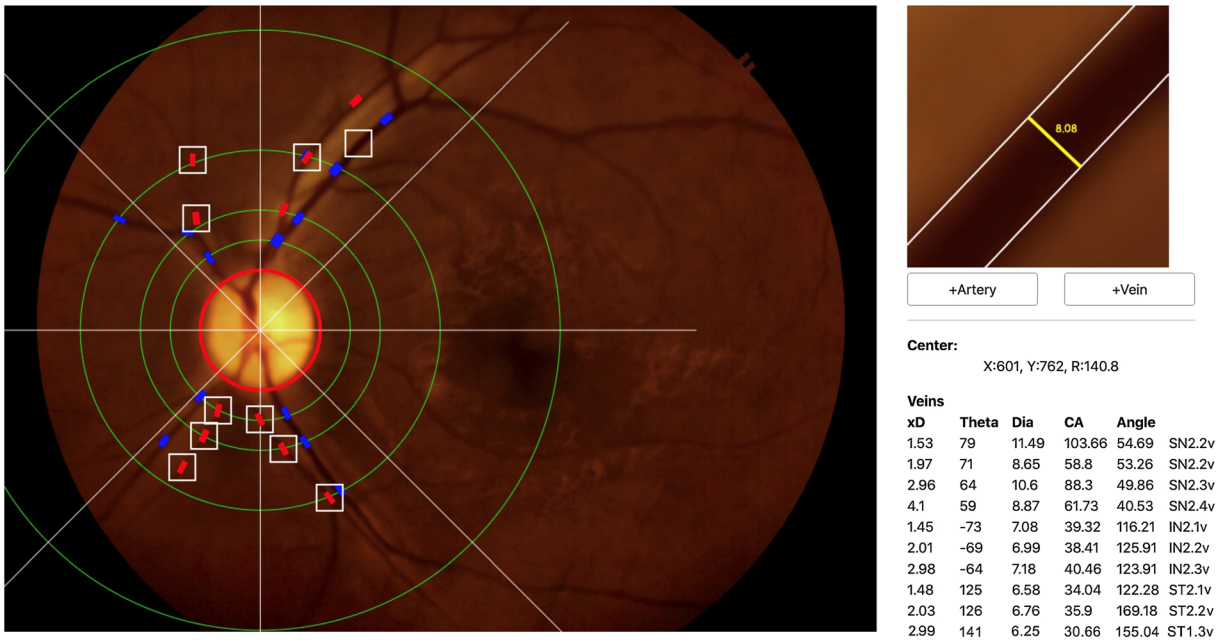

Fig. 2 Illustration from the retinal vascularity index (RVI) software demonstrating demarcation of arteries (in red) and veins (in blue) to compute the focal retinal vascularity index (ratio of area of artery to area of vein). Various RVI (AVR) points are computed for each of the zones. The callipers to identify the boundaries of retinal vessels are also illustrated in the figure

the COVID-positive cohort (39 men, 76.5% vs. 12 women, 23.5%) with a mean age of 46.5 ± 12.9 years. The subjects in the control group were significantly younger than the cohort group. There were also significantly fewer women in the COVID group as compared to the control group (Table 1).Overall, the mean RVI of all 16 points across the retina was 0.34 ± 0.02 in patients with COVID and 0.33 ± 0.02 in control subjects, with no statistically significant difference between the two groups (Table 1).

The RVI of each point at different quadrants was analysed individually (Table 2). Out of the 16 points being measured, RVI at three points, namely SN1.1, IT2.1 and IN2.1, had statistically significant greater value in COVID-19-positive patients as compared to those in normal controls.

Superonasal Quadrant

With regards to the individual focal point value, the RVI value at SN1.1 in the COVID-19-positive cohort was higher than the RVI in the control cohort with a statistically significant difference. A slightly greater RVI was also found at SN1.2 and SN2.2 in the COVID-19-positive group compared to controls. However, these differences were not statistically significant. The values in both groups at SN2.1 were nearly equal.

Superotemporal Quadrant

The RVIs of ST1.1 and ST2.2 in COVID-19-positive cohort were lower than those in the control group, even though not statistically significant. The RVIs of ST2.1 in both groups were comparable. In contrast, the RVI of ST1.2 was greater in the COVID-19-positive group compared with the control group.

Inferotemporal Quadrant

Except for RVI of IT1.1 with similar results in both groups, the rest of the RVI values in the

Table 1 Demographic characteristics and retinal vascularity indices (RVI) of the 116 Subjects (221 eyes)

Characteristics	Overall (N = 116)	Control (N = 65)	Cohort (N = 51)	p value (Control vs. cohort)
Age				
Mean (SD), years	43.8 (11.9)	41.7 (10.7)	46.5 (12.9)	0.032[a]
Gender				
Male (%)	75 (64.7)	36 (55.4)	39 (76.5)	0.018[b]
Female (%)	41 (35.3)	29 (44.6)	12 (23.5)	
Diabetes				
No (%)	90 (77.6)	57 (87.7)	33 (64.7)	0.003[b]
Yes (%)	26 (22.4)	8 (12.3)	18 (35.3)	
Hypertension				
No (%)	95 (81.9)	56 (86.2)	39 (76.5)	0.179[b]
Yes (%)	21 (18.1)	9 (13.8)	12 (23.5)	
Global RVI[c] mean ± SD (95% CI)		0.33 ± 0.02 (0.29–0.36)	0.34 ± 0.02 (0.30–0.38)	0.64

[a]Unpaired t test with equal variance
[b]Chi-square test
[c]Overall RVI (%) parameter comparison using linear mixed model with AR1 as repeated covariance, while treating age and gender, pre-existing diabetes mellitus and hypertension as covariate

COVID-19-positive group were higher than those in the control group, with a statistically significantly difference between the two groups at IT2.1

Inferonasal Quadrant

Furthermore, the RVI values at IN2.1 were significantly greater than those of controls group.

DISCUSSION

Retinal findings associated with patients with COVID-19 have been reported in a few case series (Table 3). Marinho et al. [11] reported four cases with subtle cotton wool spots and micro-haemorrhages along the retinal arcades observed on fundus examination. Hyperreflective lesions at the level of ganglion cell and inner plexiform layers were also shown in all patients from the case series (12 patients in total). Furthermore, Virgo and Mohamed [12] presented two patients with paracentral acute middle maculopathy and acute macular neuroretinopathy following COVID-19 infection. In addition, Insausti-García et al. [13] reported a case of papillophlebitis associated with SARS-CoV-2. Casagrande and associates also detected SARS-CoV-2 viral RNA in the retina of patients who had died owing to COVID-19 in their autopsy study [14]. Given the presence of ACE-2 receptors in various layers of the retina and choroid, pathoanatomical abnormalities in these ocular tissues may be expected.

It is suggested that immune-mediated vascular damage and inflammation caused by COVID-19 leads to endotheliitis, tissue oedema

Table 2 Quadrant-wise distribution of focal retinal vascularity index (RVI) values at each focal points

Focal RVI values	Control (N = 65) (125 eyes)		Cohort (N = 51) (96 eyes)		Mean difference (Control − cohort)	p value[a] (Control vs. cohort)
	Mean ± SD	(95% CI)	Mean ± SD	(95% CI)	Mean (95% CI)	
SN						
1.1r	0.26 ± 0.03	(0.20–0.32)	0.33 ± 0.03	(0.27–0.39)	− 0.07 (− 0.14 to 0.00)	0.037*
1.2r	0.34 ± 0.05	(0.24–0.44)	0.37 ± 0.05	(0.27–0.46)	− 0.03 (− 0.13 to 0.07)	0.574
2.1r	0.34 ± 0.03	(0.28–0.39)	0.33 ± 0.03	(0.28–0.39)	0.01 (− 0.06 to 0.07)	0.856
2.2r	0.33 ± 0.03	(0.27–0.38)	0.34 ± 0.03	(0.29–0.39)	− 0.01 (− 0.08 to 0.05)	0.652
ST						
1.1r	0.30 ± 0.04	(0.22–0.37)	0.27 ± 0.03	(0.20–0.34)	0.03 (− 0.06 to 0.12)	0.552
1.2r	0.28 ± 0.04	(0.20–0.36)	0.28 ± 0.03	(0.21–0.34)	0.00 (− 0.09 to 0.10)	0.980
2.1r	0.36 ± 0.02	(0.31–0.41)	0.36 ± 0.03	(0.31–0.41)	0.00 (− 0.06 to 0.05)	0.932
2.2r	0.38 ± 0.03	(0.33–0.44)	0.36 ± 0.03	(0.30–0.42)	0.02 (− 0.04 to 0.09)	0.462
IT						
1.1r	0.32 ± 0.04	(0.24–0.39)	0.33 ± 0.04	(0.25–0.40)	− 0.01 (− 0.09 to 0.07)	0.835
1.2r	0.34 ± 0.05	(0.24–0.44)	0.36 ± 0.05	(0.26–0.46)	− 0.02 (− 0.12 to 0.08)	0.717
2.1r	0.29 ± 0.02	(0.24–0.33)	0.34 ± 0.02	(0.30–0.39)	− 0.05 (− 0.11 to 0.00)	0.048*
2.2r	0.30 ± 0.02	(0.25–0.35)	0.31 ± 0.02	(0.26–0.36)	− 0.01 (− 0.07 to 0.05)	0.763
IN						
1.1r	0.42 ± 0.07	(0.28–0.55)	0.41 ± 0.07	(0.28–0.55)	0.00 (− 0.08 to 0.09)	0.981
1.2r	0.56 ± 0.11	(0.32–0.79)	0.53 ± 0.11	(0.29–0.77)	0.02 (− 0.10 to 0.14)	0.697
2.1r	0.31 ± 0.02	(0.26–0.35)	0.36 ± 0.02	(0.31–0.41)	− 0.06 (− 0.11 to 0.00)	0.049*
2.2r	0.31 ± 0.03	(0.26–0.36)	0.37 ± 0.03	(0.31–0.42)	− 0.06 (− 0.12 to 0.01)	0.078

*Significant at $p < 0.05$

[a]RVI (%) parameter comparison using linear mixed model with diagonal as repeated covariance, while treating age and gender, pre-existing diabetes mellitus and hypertension as covariate

and the activation of coagulation pathways [15]. This pathomechanism has been implicated in both pulmonary and extrapulmonary complications of COVID-19, ranging from acute myocardial infarction, ischaemic stroke, acute kidney injury, gastrointestinal damage and dermal injury [16, 17]. These numerous reports of systemic microvascular injury and thrombosis in patients with severe COVID-19 infection suggest that these processes may potentially also be occurring in the eye, highlighting the importance of evaluating retinal vascular involvement with this disease [15].

In our study, we developed a new method of retinal vascular measurement to obtain a ratio between arteriolar and venular widths as an index in different segments of retina to assess the topographical changes in the retinal

Table 3 Summary of the studies on retinal findings and retinal vasculature in patients with COVID-19

References	Summary	Retinal changes	Investigation	Ocular symptoms	Time to onset from infection	Comments
Invernizzi et al. [6] "Retinal findings in patients with COVID-19: results from the SERPICO-19 study"	Cross-sectional study reporting presence of retinal alterations in patients with COVID-19 using fundus photography	Haemorrhages, cotton wool spots, dilated veins, tortuous vessels, increased mean arterial and mean vein diameters	Fundus photography	Eye redness, eye discomfort, photophobia, decreased visual acuity	Within 30 days	
Marinho et al. [11] "Retinal findings in patients with COVID-19"	OCT evaluation of 12 patients with COVID-19 11–33 days after COVID-19 symptom onset	Hyperreflective lesions of the ganglion cell and inner plexiform layers (particularly at the papillomacular bundle in both eyes)	OCT	NA	11–33 days	No report of ocular symptoms; no further follow-up reported for changes in findings—as addressed by Vavvas et al.
Virgo and Mohamed [12] "Paracentral acute middle maculopathy and acute macular neuroretinopathy following SARS-CoV-2 infection"	Case report of paracentral scotoma following SARS-CoV-2 infection	Focal hyperreflective change in inner and outer plexiform layers with inner nuclear layer volume loss, disruption of interdigitation zone	OCT	Paracentral scotoma	16–35 days	Both patients have history of acephalgic visual migraine aura and one had history of right toxoplasma chorioretinitis

Table 3 continued

References	Summary	Retinal changes	Investigation	Ocular symptoms	Time to onset from infection	Comments
Insausti-García et al. [13] "Papillophlebitis in a COVID-19 patient: inflammation and hypercoagulable state"	Case report of papillophlebitis in a COVID-19-infected patient	Dilated and tortuous retinal vessels, disc oedema, retinal haemorrhages, macular oedema	Fluorescein angiography (FA), OCT	Visual field diffuse decrease in sensitivity, slight central scotoma, moderate increase in blind spot, decrease in visual acuity	6 weeks	No diagnostic test to confirm COVID-19, though patient had flu-like symptoms that coincided with the coronavirus pandemic onset in Madrid, Spain
Landecho et al. [30] "COVID-19 retinal microangiopathy as an in vivo biomarker of systemic vascular disease"	Case series of 27 asymptomatic COVID-19 subjects	Cotton wool spots (unilateral and bilateral)	OCT with B scan, OCT-angiography, fundoscopy	NA	30–58 days	
Abrishami et al. [31] "Optical coherence tomography angiography analysis of the retina in patients recovered from COVID-19: a case–control study"	Quantification of the density of macular microvasculature and the area of the foveal avascular zone in 31 patients with COVID-19 and 23 healthy controls	Macular superficial retinal capillary plexus and deep retinal capillary plexus significantly reduced in COVID-19 cohort	OCT-angiography (OCT-A), B scan	NA	At least 2 weeks	Foveal avascular zone greater in COVID-19 group though found to be not statistically significant

Table 3 continued

References	Summary	Retinal changes	Investigation	Ocular symptoms	Time to onset from infection	Comments
Gascon et al. [24] "COVID-19 associated retinopathy: a case report"	Case report of acute macular neuroretinopathy and paracentral acute middle maculopathy	Perifoveal vascular remodelling, retinal haemorrhages (including Roth spots), discrete optic disc staining without vasculitis, hyperreflective bands of inner nuclear layer, Henle fibre layer and outer plexiform layer, disruption of ellipsoid and interdigitation zone, subretinal fluid	FA, indocyanine green angiography (ICG), OCT	Decrease in visual acuity, dyschromatopsia, scotoma	8 days	Right eye loss of vision from traumatic glaucoma
Sawalha et al. [32] "COVID-19 induced acute bilateral optic neuritis"	Case report of bilateral optic neuritis in a COVID-19 patient	Visual field defects, RAPD, MRI enhancement of optic nerve	MRI brain, lumbar puncture	Decrease in visual acuity and eye pain	2 weeks	Patient had anti-MOG antibodies—possibly an activation by the COVID-19 virus instead
Lani-Louzada et al. [33] "Retinal changes in COVID-19 hospitalized cases"	Case series of retinal findings in severely ill patients with COVID-19	Nerve fibre layer infarcts, retinal haemorrhages	Fundus photography	NA	12–59 days	Patients studied were septic which may confound retinal findings

Table 3 continued

References	Summary	Retinal changes	Investigation	Ocular symptoms	Time to onset from infection	Comments
Walinjkar et al. [34] "Central retinal vein occlusion with COVID-19 infection as the presumptive etiology"	Case report of a COVID-19 patient with central retinal vein occlusion	Disc oedema, retinal haemorrhages, cystoid macular oedema, neurosensory retinal detachment	Fundus photography, OCT	Decrease in visual acuity	21 days	Subject has history of polycystic ovaries not on medications, no confirmation of COVID-19 serology despite CT lung findings
Ortiz-Seller et al. [35] "Ophthalmic and neuro-ophthalmic manifestations of coronavirus disease 2019 (COVID-19)"	Case report of inflammatory chorioretinopathy and Adie's syndrome in COVID-19 patient	Adie tonic pupil, choriocapillaris nonperfusion, disruption of ellipsoid and interdigitation zone, chorioretinal lesions	Fundus autofluorescence, OCT-A, OCT	Retroocular pain, decreased visual acuity	2 days	

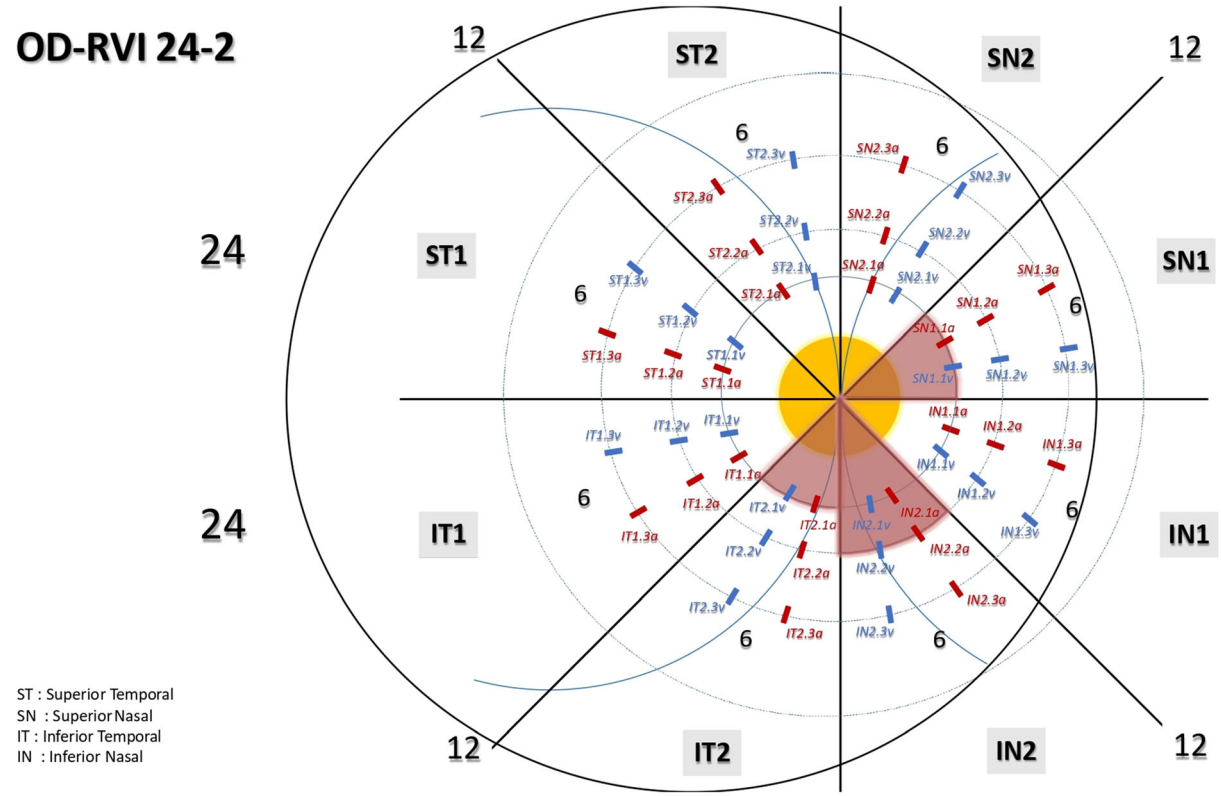

Fig. 3 Diagrammatic illustration of the retinal vascularity index (RVI) topographical map showing the areas of abnormal RVI values

vasculature. The development was used to assess the retinal microvascular change in patients with COVID. To our knowledge, this is the first study to utilise semi-automated software to analyse the AVR in focal points in different quadrant of the retina, as some studies suggest that the vascular perfusion and retinal vessel oxygen saturation in different quadrants might vary [16]. Hence, there is value in evaluating AVR in various points in different locations of retina.

In our study, we were not able to establish an obvious impact of COVID-19 infection on retinal vasculature, which could be explained by comparably dilated arterioles and venules or the lack of disease severity grading. The overall median RVI value was slightly greater in the COVID group; however, the difference between the two groups was not statistically significant. It is noteworthy that our study did not evaluate the arteriolar and venular calibers separately. Invernizzi et al. demonstrated in their study both retinal arterioles and veins were larger in COVID-positive patients compared to unexposed subjects, but they did not measure AVR which is a common indicator in systemic diseases affecting retinal microvasculature, such as hypertension and cardiovascular disease [6]. The reason we chose AVR to be our primary outcome was based on the evidence shown in various studies that COVID-19 infection can cause endothelial damage and thrombotic events.

With regards to segmentation of RVI in different quadrants, it was an effort to develop a novel retinal vascular parameter to assess the localised vascular features. Out of the 16 points being measured, there were three points in the map which detected a statistically significant greater value of RVI in the COVID group as compared to the control group (Fig. 3). Even though the clinical relevance of these findings was unclear, it might suggest an advantage of obtaining topographic parameters of retinal

vasculature as changes associated with systemic microvascular diseases are probably focal alterations such as arteriovenous nicking. There are presently other novel technologies under investigation to examine the focal geometry and topographic retinal vascular features, including the branching angles of blood vessels, retinal vessel tortuosity and fractal dimension [17].

A possible mechanism for focal microangiopathy could be local retinal pericyte drop-out. Pericytes of the retina are known to affect vascular remodelling and have been proposed as the inciting event for early diabetic changes in the eye [18]. Depletion of pericytes induce inflammatory changes in murine retinal vessels, particularly the endothelial cells [19]. In murine brain models, pericytes function as early sensors of systemic inflammation and secrete immune-mediating molecules such as interleukin-33 (IL-33) and C-X3-C motif chemokine ligand (CX3CL1) [20–22]. These have been shown to promote anti-inflammatory microglial response in murine models [23]. Jidigam et al. reported increased microglial cells in the retina in a study of post-mortem COVID-19 eyes, suggesting that microglial dystrophy could contribute to the secretion of pro-inflammatory molecules, resulting in retinopathy [24]. Furthermore, Park et al. studied murine models of selective pericyte loss and found sensitisation of retinal endothelial cells to vascular endothelial growth factors in a positive feedback cycle that is similar to the pathogenesis of diabetic retinopathy [25]. These lend credence to our hypothesis for focal microangiopathy.

An alternative mechanism may be a local hypercoagulable state induced by the virus. Of note, several clinical reports of lung autopsies have demonstrated thrombotic microangiopathy (TMA) in patients with COVID-19 [26, 27]. Local retinal COVID-19 replication may have contributed to the focal endothelial injury of the retina via a complement cascade, similar to that in diabetic retinopathy [28]. The complement cascade, as part of the innate immune system, is part of the earliest host defensive response against pathogens, including RNA viruses. Magro et al. found extensive complement component deposition within the lung microvasculature of patients with COVID-19 [15]. Ackermann et al. [26] further reported the finding of enhanced intussusceptive angiogenesis in lungs of patients with COVID-19. The finding of an intussusceptive pillar characteristic of the condition is not readily available on light microscopy (as with the indirect ophthalmoscope) and requires the use scanning electron microscopy and corrosion casting [29]. This may contribute to the focal retinal findings in our study.

Limitations of the study should also be noted. Firstly, the individual diameters of retinal arterioles and venules could have been analysed separately, which may be helpful in determining the microvascular changes caused by the COVID-19 infection. Secondly, we had a relatively small sample size as this was a pilot study, and thus sample size was not calculated. The smaller sample size may have limited the power of our statistical analysis, and future studies with larger sample sizes will be needed to confirm the findings of this pilot study. In addition, we did not collect data on or further subgroup the patients according to the severity of their COVID-19 infection. Finally, assessing findings of fundus images such as microaneurysms, retinal haemorrhages and cotton wool spots together with retinal vascular calibers and architecture will assist in establishing RVI's applicability in the clinical setting.

CONCLUSION

We were not able to ascertain an influence of COVID-19 on retinal vascular alterations based on RVI results. However, localised greater RVI values were found in some of the points, which indicates a likely more focal change of the vasculature. Nonetheless, the lack of association suggests the mechanism of retinal involvement in COVID is still unclear and requires more study. It also prompts the further development

of different architectural retinal vascular parameters to consolidate the use of retinal vascular indices in evaluating various types of retinal microangiopathy.

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Data Availability. The data sets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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