NEUROPHTHALMOLOGY

The effect of optic neuritis attacks on choroidal vascularity index in patients with multiple sclerosis

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Abstract

Purpose To investigate the changes in the choroidal vascularity index (CVI) of patients with multiple sclerosis (MS) using binarization on enhanced depth imaging optical coherence tomography (EDI-OCT) images and to evaluate the effect of optic neuritis (ON) attacks on these measurements.

Method Three groups were created by including forty eyes of 20 patients diagnosed with relapsing-remitting MS and had a unilateral history of ON attack and the randomly selected eyes of 30 healthy age- and sex-matched control subjects. Group 1 ($n = 20$) consisted of the ON-affected eyes of the MS patients (MSON); group 2 ($n = 20$) included their fellow healthy eyes (MSNON); group 3 ($n = 30$) included the eyes of healthy controls.

Results The mean age was 33.3 ± 9.4 years in the MS group and 33.4 ± 11.1 years in the healthy control group. Mean choroidal vascularity index (CVI) was significantly lower in the MSON group than the MSNON group (59.6 ± 3.72 % vs 61.7 ± 3.16 %, $p = 0.007$). The CVI values of both the MSON and MSNON groups were significantly lower when compared to the controls (63.9 ± 2.76) ($p < 0.001$, $p = 0.030$). Compared to controls, the subfoveal total choroidal area and luminal area values were significantly greater in the MSON ($p = 0.009$, $p = 0.009$, respectively) and MSNON groups ($p = 0.031$, $p = 0.013$, respectively).

Conclusion The presented study demonstrates that, compared to healthy subjects, CVI values are lower in the affected and unaffected eyes of patients who had a history of ON in relation with their MS diagnosis. The presence of significant anatomical changes, especially in the luminal area, may suggest that ON causes vascular disorganization which contributes to MS pathophysiology.

Key Messages

- The dense vascular choroid layer is affected by disorders that cause systemic inflammation and vascular disorganization, such as multiple sclerosis.

- In recent years, EDI-OCT images have been binarized utilizing a software program to separate the choroid into luminal and interstitial areas in order to measure the vascularity changes in the choroid more precisely.

- To the best of our knowledge, this study studied choroidal vascularity index for the first time in patients with multiple sclerosis who had a unilateral history of optic neuritis attacks. Our results demonstrate that choroidal vascularity index was significantly lower in eyes affected by optic neuritis attacks when compared to unaffected fellow eyes and healthy control eyes. A moderate negative correlation was found between the Expanded Disability Status Scale and the choroidal vascularity index values of eyes affected by optic neuritis. This value, which is an indicator of structural changes in the choroidal layer, might also be a useful prognostic factor in patients with MS.

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Introduction

Multiple sclerosis (MS) is a chronic inflammatory and degenerative disease of the central nervous system. It is one of the most common neurological disorders, affecting an estimated 2.3 million people worldwide [1]. The disease often occurs in young adults. While some types are characterized by attacks, others are progressive [2]. The most common ocular symptom of the disease is optic neuritis (ON), which is caused by axonal degeneration of the optic nerve. This problem manifests itself as the first clinical sign of the disease in 25% of patients and is seen in 70% of patients during the disease [3].

Autoimmunity plays a prominent role in the pathogenesis of MS [4]. More specifically, demyelination and neurodegeneration have been associated with T cell-mediated inflammation in the central nervous system [5]. In the context of ocular findings, it has been shown that retinal nerve fiber layer thickness (RNFLT) is decreased due to axonal damage in patients with MS [6, 7]. Furthermore, vascular disorganization mechanisms are known to be involved in the pathophysiology of MS. Some Doppler ultrasonography studies have shown significantly decreased blood flow in the posterior ciliary artery and there have been reports of altered retrobulbar hemodynamics in the case of ON due to MS [8, 9]. Also, decreased extraocular blood flow and increased endothelin-1 plasma levels have been identified in patients with MS [10].

The dense vascular choroid layer is a structure that is affected by disorders characterized by systemic inflammation and vascular disorganization—such as MS [11]. It is possible to visualize the structure of the choroid and measure subfoveal choroidal thickness (SFCT)—an indicator of subfoveal ocular perfusion—in the EDI (enhanced depth imaging) mode of spectral-domain optical coherence tomography (OCT) devices [12, 13]. In recent years, EDI-OCT images have been binarized utilizing a software program to separate the choroid into luminal and interstitial areas in order to measure the vascularity changes in the choroid more precisely [14]. A quantitative value, called choroidal vascularity index (CVI), that can be identified in a semiautomated manner, emerged as a parameter that could be used to assess the vascular status of the choroid using binarization techniques [15].

This study presents the comparison of ON-affected eyes of MS patients, their fellow eyes, and healthy control eyes in terms of CVI values determined by the binarization of EDI-OCT images. To the best of our knowledge, this is the first study evaluating CVI values in MS patients with unilateral ON attack history.

Methods

Study population

This prospective study included 40 eyes of 20 patients who applied to the ophthalmology clinic and diagnosed with relapsing-remitting MS according to the McDonald criteria [16]. These patients had unilateral ON history (optic papillitis/retrobulbar neuritis). Additionally, 30 healthy eyes from a randomly selected group of age- and sex-matched healthy controls were included in the study. Three groups were created: group 1 ($n = 20$) consisted of the affected eyes (ON attack) of the MS patients (MSON group), group 2 ($n = 20$) included their fellow unaffected eyes (MSNON group), and group 3 ($n = 30$) included eyes of healthy controls. The study was conducted according to the principles put forth by the Helsinki Declaration and received ethics approval from the local clinical trials ethics committee.

Inclusion and exclusion criteria

For the MS group, the inclusion criteria were as follows: having a history of ON attack that was proven with visual field, contrasted orbital magnetic resonance imaging and examination results, attending follow-up at the Neurology and the Ophthalmology clinics, having a best-corrected visual acuity (BCVA) of ≥ 2/10, having spherical refractive errors in the range of –4 to +3 diopters and cylindrical refractive errors of ≤ 3 diopters, not having any ocular or systemic diseases other than MS, not having a history of any ocular surgery, not having a history of ON attacks as well as MS attacks for at least 3 months, and not having a history of systemic or topical cortisone use for at least 3 months.

Healthy controls were randomly selected from individuals who had applied for routine ophthalmological examination to the ophthalmology department. The inclusion criteria were as follows: the absence of any ophthalmologic pathology other than refraction errors or cataract (spherical refractive errors of –4 to +3 diopters, and cylindrical refractive errors of ≤ 3 diopters), having a BCVA of ≥ 6/10, absence of systemic diseases or a history of any ocular surgery, and not receiving any systemic or topical medications within the last 3 months.

Fig. 1 Illustration of binarized EDI-OCT images in an eye affected with ▶ optic neuritis attack in a patient with multiple sclerosis. a Enhanced depth imaging optical coherence tomography (EDI-OCT). b Binarized EDI-OCT image showing the segmentation of choroidal structures. c In the ROI manager, the first automatically calculated value represents total choroidal area and the second calculated value represents luminal area. Stromal area was calculated by subtracting luminal area from total choroidal area, and CVI was computed by dividing luminal area by total choroidal area
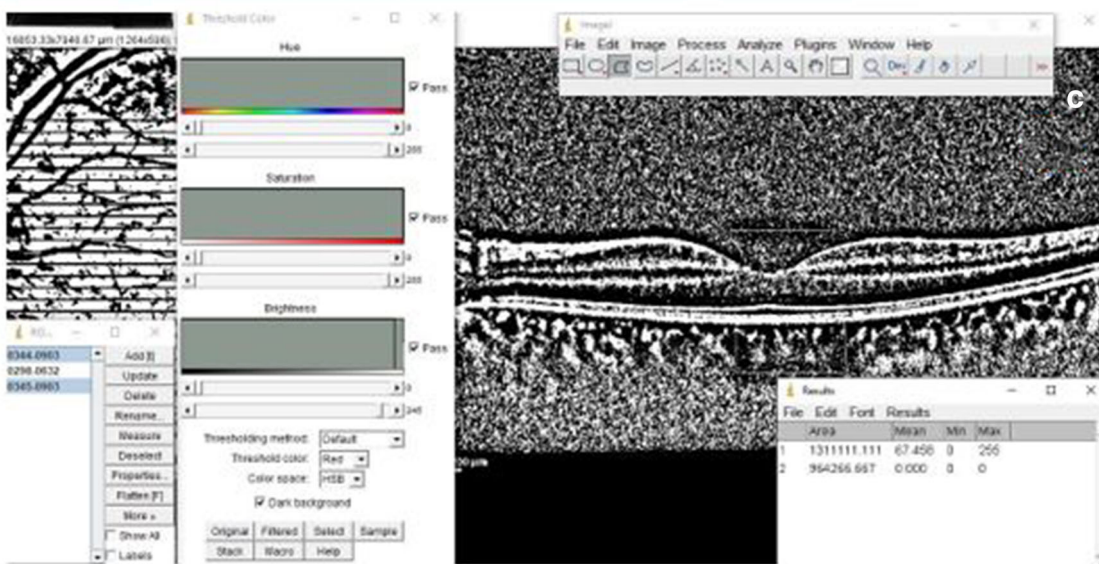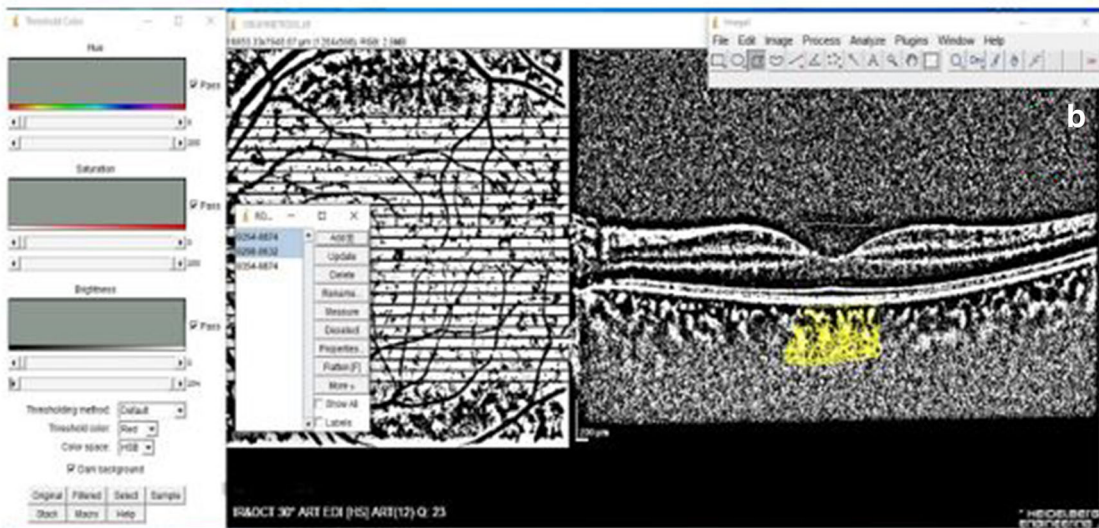
Exclusion criteria included the presence of glaucomatous damage to the optic disc in fundus examination, having glaucoma or ocular hypertension, family history of glaucoma, having optical media opacities (dense cataracts, corneal opacity, pupillary anomaly, vitreous opacity) which could affect OCT images, having uveitis or any type of diagnosed retinal pathology, and having a history of ocular surgery or major surgery involving any other system or organ.

Ophthalmologic examination, OCT imaging, and measurement protocols

All participants underwent the Snellen BCVA test, biomicroscopic anterior segment examination, IOP measurement with Goldmann applanation tonometer (Haag–Streit, Bern, Switzerland), axial length (AXL) measurement (IOL Master, Carl Zeiss Meditec, Dublin, CA), central corneal thickness (CCT) measurement (Topcon, RK-8800, Tokyo), pupillary reflexes, eye movements, and fundus examination. All participants underwent OCT (Heidelberg Engineering

GmbH, Heidelberg, Germany) examination, and the Humphrey visual field (Humphrey Visual Field Analyzer III, Carl Zeiss Inc., Dublin, CA) was performed with 24-2 SITA standard strategy for MS patients. The OCT scans were performed from 9 AM to 11 AM in order to minimize diurnal variation of choroid thickness. All scans were performed with a target of sustaining high (> 50) signal strength index scores.

We utilized the standard settings for the EDI mode of OCT imaging: 1024 A-scans on a 6-mm horizontal line passing through the center of the fovea were obtained from all participants. Subfoveal choroidal thickness was measured with horizontal B-scans on vertical lines running from the retinal pigment epithelium's outer border towards the choroidoscleral junction in the subfoveal region. Measurements were performed using the manual measurement tool of the OCT device by two independent observers blinded to the clinical characteristics of patients.

The EDI-OCT images (Fig. 1a) were binarized in the Image J software (Version 1.52i, National Institutes of

Table 1 Compared characteristics and measurement parameters between the three groups

	Group 1 (MSON) Mean±sd	Group 2 (MSNON) Mean±sd	Group 3 (HC) Mean±sd	p
Gender; n (%) F/M	7(35)/13(65)	7(35)/13(65)	12(40)/18(60)	0.958
Age	33.25±9.44	33.25±9.44	33.4±11.14	0.999
IOP	13.66±1.96	13.8±1.97	12.83±1.32	0.236
CCT (μm)	559.55±10.3	562.65±15.26	570.83±19.92	0.174
Refractive error				
Spherical (diopter)	−0.35±0.59	−0.7±0.64	−0.14±0.87	0.347
Cylindrical (diopter)	−0.31±0.57	−0.53±0.69	−0.31±0.5	0.093
Axis	53.05±66.26	67.65±66.12	62.63±80.3	0.562
AXL (mm)	23.54±0.33	23.54±0.35	23.52±0.32	0.981
BCVA (Snellen)	0.67±0.33	1±00	1±0	**<0.001****
SFCT (μm)	281.75±81.89	308.3±101.15	286.23±57.14	0.322
T (μm)	72.8±15.88	69.35±20.11	80.6±14.47	0.142
ST (μm)	132.55±26.74	126.9±30.45	142.5±30.24	0.304
IT (μm)	145.15±20.25	139.85±25.13	156.7±17.3	0.053
N (μm)	73.8±17.15	77.75±27.96	80.77±27.4	0.582
SN (μm)	110.35±23.02	106±22.87	111.87±27.64	0.551
IN (μm)	106±23.86	106.65±24.96	113.63±24.33	0.621
RNFLT (μm)	97.3±17.91	99.1±16.69	105.73±12.84	0.261
CMT (μm)	262.75±12.8	264.85±14.59	264.23±18.13	0.703
CMV (mm³)	0.21±0.01	0.21±0.01	0.21±0.01	0.873
EDSS	3.05±1.33	3.15±1.28		
CVI (%)	59.6±3.72	61.7±3.16	63.9±2.76	**<0.001****
TCA (mm²)	1.95±0.56	1.87±0.55	1.5±0.51	**0.029***
SA (mm²)	0.75±0.31	0.72±0.25	0.68±0.22	0.101
LA (mm²)	1.21±0.34	1.20±0.35	0.92±0.34	**0.022***

GLMM (generalized linear mixed models)

p values are the result of 3-group comparisons of the respective parameters described in the rows

*p < 0.05 **p < 0.01

Bold p values are statistically significant

MSON; the eyes affected with optic neuritis attack in patients with multiple sclerosis, MSNON; their healthy fellow eyes, HC; healthy controls, F; female, M; male, IOP; intraocular pressure; CCT; central corneal thickness, AXL; axial length, BCVA; best-corrected visual acuity, SFCT; subfoveal choroidal thickness, T; temporal, ST; superotemporal, IT; inferotemporal, N; nasal, SN; superonasal, IN; inferonasal, RNFLT; retinal nerve fiber layer thickness, CMT; central macular thickness, CMV; central macular volume, EDSS; expanded disability status scale, CVI; choroidal vascular index, TCA; total choroidal area, SA; stromal area, LA; Luminal area

Table 2 Relationships between variables in eyes affected with ON attack in patients with multiple sclerosis

		Age	SFCT	ON time	DD	RNFLT	CMT	CMV	EDSS	CVI	TCA	SA
SFCT	r	-0.281	-									
	p	0.230	-									
ON time	r	0.451	-0.015	-								
	p	**0.046***	0.951	-								
DD	r	0.567	-0.124	0.619	-							
	p	**0.009****	0.601	**0.004****	-							
RNFLT	r	-0.219	-0.255	-0.435	-0.260	-						
	p	0.353	0.279	0.055	0.269	-						
CMT	r	0.114	0.137	-0.106	-0.120	0.073	-					
	p	0.632	0.565	0.656	0.614	0.761	-					
CMV	r	0.126	0.307	-0.030	-0.062	-0.124	0.876	-				
	p	0.596	0.189	0.899	0.795	0.601	**<0.001****	-				
EDSS	r	0.158	0.418	0.269	0.257	0.027	0.299	0.153	-			
	p	0.506	0.066	0.251	0.273	0.911	0.200	0.520	-			
CVI	r	0.070	-0.155	-0.049	0.092	0.263	-0.084	-0.251	-0.407	-		
	p	0.768	0.515	0.837	0.699	0.263	0.726	0.286	**0.002****	-		
TCA	r	-0.142	0.353	0.039	-0.305	-0.173	-0.101	0.058	-0.038	-0.338	-	
	p	0.549	0.127	0.871	0.191	0.465	0.673	0.809	0.874	0.145	-	
SA	r	-0.187	0.451	0.150	-0.104	-0.152	-0.355	-0.173	-0.072	-0.304	0.819	-
	p	0.430	**0.046***	0.528	0.663	0.523	0.125	0.466	0.763	0.192	**<0.001****	-
LA	r	-0.033	0.170	-0.088	-0.410	-0.147	0.171	0.274	-0.055	-0.272	0.854	0.409
	p	0.890	0.474	0.713	0.073	0.535	0.472	0.242	0.817	0.246	**<0.001****	0.073

Pearson correlation analysis $*p < 0.05**p < 0.01$

Bold p values are statistically significant

ON; optic neuritis, SFCT; subfoveal choroidal thickness, DD; disease duration, RNFLT; retinal nerve fiber layer thickness, CMT; central macular thickness, CMV; central macular volume, EDSS; expanded disability status scale, CVI; choroidal vascular index, TCA; total choroidal area, SA; stromal area, LA; luminal area

Health, Bethesda, MD USA) using the protocol described by Agrawal et al. [15] (Fig. 1b). The subfoveal area from the retinal pigment epithelium to the choroidoscleral junction was marked with the polygon selection tool from 750-μm nasal and 750-μm temporal to the fovea. The marked total choroidal area image was binarized using Niblack's autolocal thresholding method. The image was converted to 8 bits and was applied an autolocal threshold; the luminal area was highlighted by first converting the image to red-green-blue color space and then applying the color threshold. The first automatically calculated value represents total choroidal area in the ROI manager, and the second calculated value represents luminal area (Fig. 1c). Stromal area is calculated by subtracting luminal area from total choroidal area, and CVI is computed by dividing luminal area by total choroidal area. All CVI procedures were repeated three times by the same observer who was blinded to patients' diagnosis, and the average of these three measurements was recorded and used in the analyses.

The peripapillary RNFLT was measured around the disc with 16 averaged circular B-scans (12° in diameter) and automatically segmented using the SPECTRALIS software. Then, patients' average peripapillary RNFLT was recorded.

Central macular thickness and volume were measured at 1-mm diameter fovea area of the topographic thickness map in 9 zone maps defined by the Early Treatment Diabetic Retinopathy Study.

Statistical method

Statistical analysis was performed using the Number Cruncher Statistical System (NCSS) 2007 (Kaysville, UT, USA) program. Descriptive statistical methods (mean, standard deviation, frequency, percentage, minimum, maximum) were used when depicting study data. The Shapiro-Wilk test and graphical examinations were used to test the suitability of quantitative data to normal distribution. Generalized linear mixed models were used for comparisons between groups and to determine the factors affecting the presence of ON. Pearson correlation analysis was used to evaluate the relationships between quantitative variables. Univariate and multivariate regression models were used to determine the effect of the

Table 3 Relationships between variables in eyes unaffected with ON attack in patients with multiple sclerosis

		SFCT	DD	RNFLT	CMT	CMV	EDSS	CVI	TCA	SA
DD	r	-0.375	-							
	p	0.104	-							
RNFLT	r	0.248	-0.100	-						
	p	0.292	0.676	-						
CMT	r	-0.112	0.016	-0.037	-					
	p	0.639	0.948	0.878	-					
CMV	r	0.108	-0.129	0.062	0.670	-				
	p	0.652	0.588	0.795	**0.001****	-				
EDSS	r	0.185	0.290	0.246	0.115	0.118	-			
	p	0.436	0.216	0.296	0.628	0.619	-			
CVI	r	-0.074	0.216	0.024	-0.185	-0.325	-0.040	-		
	p	0.757	0.360	0.922	0.434	0.162	0.867	-		
TCA	r	0.676	-0.341	0.279	-0.099	-0.038	0.303	-0.351	-	
	p	**0.001****	0.141	0.234	0.679	0.872	0.194	0.129	-	
SA	r	0.604	-0.297	0.234	-0.097	-0.009	0.271	-0.494	0.979	-
	p	**0.005****	0.203	0.320	0.685	0.970	0.249	0.027	**<0.001****	-
LA	r	0.648	-0.387	0.073	-0.244	-0.229	0.107	-0.134	0.855	0.795
	p	**0.002****	0.092	0.759	0.301	0.331	0.654	0.574	**<0.001****	**<0.001****

Pearson correlation analysis *p < 0.05**p < 0.01

Bold *p* values are statistically significant

ON; optic neuritis, SFCT; subfoveal choroidal thickness, DD; disease duration, RNFLT; retinal nerve fiber layer thickness, CMT; central macular thickness, CMV; central macular volume, EDSS; expanded disability status scale, CVI; choroidal vascular index, TCA; total choroidal area, SA; stromal area, LA; luminal area

evaluated parameters on the presence of ON and the effects of CVI values. Inter-examiner and intra-examiner intraclass correlation coefficient (ICC) was used to determine the agreement between observers. Statistical significance was accepted as $p < 0.05$.

Results

There were 20 patients (13 women, 7 men; average age: 33.25 ± 9.44 years) in the MS group and 30 patients (18 women, 12 men; average age: 33.4 ± 11.14 years) in the healthy subject group. There was no statistically significant difference between subjects with MS and healthy controls regarding age and gender distribution ($p = 0.999$ and $p = 0.958$, respectively). The mean disease duration was 53.15 ± 50.38 (12–240) months in patients with MS. The mean time that had surpassed after the last ON attack was 24.60 ± 18.16 (8–72) months before OCT shots. There were also no statistically significant differences between the groups with regard to refraction value, IOP, CCT, and AXL values. The distribution of demographic characteristics by group is given in detail in Table 1.

Mean SFCT was 281.75 ± 81.89 μm in the MSON group, 308.3 ± 101.15 μm in the MSNON group, and 286.23 ± 57.14 μm ($p = 0.322$) in the control group. The mean CVI value was 59.6 ± 3.72 in the MSON group, 61.7 ± 3.16 in the MSNON group, and 63.9 ± 2.76 in the control group. The mean CVI was found to be significantly lower in both the MSON and MSNON groups when compared with the controls ($p < 0.001$ and $p = 0.030$, respectively). In the MSON group, mean CVI was significantly lower compared to the MSNON group ($p = 0.007$). In terms of total choroidal area, both of the MSON and MSNON groups had significantly higher values compared with controls ($p = 0.009$ and $p = 0.031$, respectively). Additionally, mean total choroidal area was significantly greater in the MSON group compared to that of the MSNON group ($p = 0.047$). Mean luminal area was 1.21 ± 0.34 mm^2 in the MSON group and 1.20 ± 0.35 mm^2 in the MSNON group ($p = 0.105$), while it was found to be significantly lower in controls (0.92 ± 0.34 mm^2) compared to both of these groups ($p = 0.009$ and $p = 0.013$, respectively) (Table 1).

The mean peripapillary RNFLT value was found to be lowest in the MSON group (97.3 ± 17.91 μm); however, a significant difference was not observed between the three groups ($p = 0.261$) (Table 1).

Pearson correlation analysis results in patients with MS are shown in Table 2 and Table 3. It was determined that there is a

Table 4 Regression analysis of the factors affecting the CVI value in eyes with MSON

	Univariable		Multivariable	
	Beta (95% CI)	p	Beta (95% CI)	p
Gender (*)	-0.901 (-4.071, 2.269)	0.558	-1.563 (-4.461, 1.334)	0.270
Age (*)	0.013 (-0.153, 0.179)	0.868	-0.038 (-0.192, 0.115)	0.605
DD	0.013 (-0.017, 0.043)	0.360		
ON Time	-0.01 (-0.111, 0.091)	0.837		
IOP	-0.305 (-1.09, 0.48)	0.425		
Spherical	1.825 (-0.757, 4.406)	0.155		
Cylindrical	0.959 (-1.642, 3.559)	0.449		
BCVA	1.518 (-3.995, 7.03)	0.570		
SFCT	2.585 (0.255, 4.825)	0.156		
T	0.04 (-0.057, 0.136)	0.401		
ST	-0.023 (-0.08, 0.035)	0.419		
IT	0.012 (-0.065, 0.089)	0.744		
N	0.004 (-0.088, 0.095)	0.936		
SN	-0.048 (-0.112, 0.016)	0.132		
IN	-0.003 (-0.069, 0.063)	0.923		
RNFLT	0.004 (-0.089, 0.098)	0.922		
CMT	-0.046 (-0.166, 0.075)	0.434		
EDSS	-6.152 (-11.511, -0.793)	**0.027***	-7.074 (-12.867, -1.282)	**0.027***
TCA	-2.584 (-0.253, -4.915)	**0.032***	-2.665 (0.268, 5.063)	**0.032***
SA	-0.096 (-1.276, 1.084)	0.867		
LA	-1.21 (-5.644, 3.225)	0.574		
			Intercept: 69.055	R^2_{adj}:0.181

Bold p values are statistically significant

MSON; the eyes affected with optic neuritis attack in patients with multiple sclerosis. DD; disease duration, ON; optic neuritis, IOP; intraocular pressure; SFCT; subfoveal choroidal thickness, T; temporal, ST; superotemporal, IT; inferotemporal, N; Nasal, SN; superonasal, IN; inferonasal, RNFLT; retinal nerve fiber layer thickness, CMT; central macular thickness, EDSS; expanded disability status scale, CVI; choroidal vascular index, TCA; total choroidal area, SA; stromal area, LA; luminal area

moderate significant negative correlation between CVI values and EDSS score ($r = -0.407$, $p = 0.002$) in the MSON group. A univariate regression analysis was performed by including the parameters evaluated in the study to determine those that had an effect on CVI value. The results showed that total choroidal area and EDSS score in the MSON group (Table 4) and the total choroidal area in the MSNON group (Table 5) were predictive parameters associated with lower CVI values. In the MSON group, multivariable regression showed that, when controlled for gender and age, an increase of 1 unit in the EDSS score would cause a decrease of 7.074 units in CVI value [Beta (95% CI) = -7.074 (-12.867, -1.282), $p=0.020$]. The univariate evaluations showed that none of the variables had a statistically significant effect on the presence of ON in the MSON group ($p > 0.05$).

There was a good agreement between intra-observer and inter-observer measurements of SFCT values (ICC = 0.999, p <0.001). Figure 2 shows color fundus photo (a), OCT image of RNFLT map (b), and 24-2 SITA Humphrey visual field (c)

for the left affected eye (ON attack, 18 months prior to scan) in a patient with MS.

Discussion

In this study, CVI values were found to be significantly lower (and total choroidal area was expanded) in the ON-affected eyes of MS patients, when compared to their unaffected eyes and healthy control eyes. Additionally, the unaffected eyes of patients with MS also demonstrated significantly lower mean CVI values and significantly higher total choroidal area and luminal area values compared to the control group. Nevertheless, there was no significant difference between the three groups regarding mean SFCT.

The dense vascular choroid layer is affected by disorders that cause systemic inflammation and vascular disorganization, such as MS [11]. The choroid supplies blood flow to the retinal pigmented epithelium, photoreceptors, and the prelaminar portion of the optic nerve [17–19]. Since the

Table 5 Regression analysis of the factors affecting the CVI value in eyes with MSNON

	Univariable		Multivariable	
	Beta (95% CI)	p	Beta (95% CI)	p
Gender (*)	1.582 (-2.099, 5.263)	0.378	1.833 (-1.560, 5.225)	0.269
Age (*)	0.028 (-0.167, 0.222)	0.768	0.025 (-0.150, 0.201)	0.762
DD	0.007 (-0.029, 0.043)	0.699		
IOP	-0.403 (-1.318, 0.512)	0.367		
Spherical	2.584 (0.253, 4.915)	0.052		
Cylindrical	1.935 (-0.795, 4.665)	0.154		
SFCT	2.518 (1.995, 7.03)	0.570		
T	0.04 (-0.049, 0.13)	0.356		
ST	0.017 (-0.042, 0.077)	0.549		
IT	0.05 (-0.019, 0.119)	0.143		
N	0.031 (-0.033, 0.095)	0.322		
SN	-0.021 (-0.101, 0.059)	0.592		
IN	0.009 (-0.065, 0.083)	0.801		
RNFLT	0.055 (-0.045, 0.154)	0.263		
CMT	-0.021 (-0.147, 0.104)	0.726		
EDSS	0.554 (-0.86, 1.968)	0.421		
TCA	-2.257 (-5.366, 0.852)	**0.034***	-2.655 (0.255, 5.075)	**0.034***
SA	-3.684 (-9.396, 2.029)	0.192		
LA	-2.979 (-8.195, 2.237)	0.246		
			Intercept: 58.961	R^2_{adj}:0.161

Bold *p* values are statistically significant

MSNON; the eyes unaffected with optic neuritis attack in patients with multiple sclerosis

DD; disease duration, IOP; intraocular pressure; SFCT; subfoveal choroidal thickness, T; temporal, ST; superotemporal, IT; inferotemporal, N; nasal, SN; superonasal, IN; inferonasal, RNFLT; retinal nerve fiber layer thickness, CMT; central macular thickness, EDSS; expanded disability status scale, CVI; choroidal vascular index, TCA; total choroidal area, SA; stromal area, LA; luminal area

choroid demonstrates considerable changes in several diseases, choroidal imaging has become essential to understand the pathophysiology of such diseases and their effects in the eye. The EDI-OCT technique introduced by Spaide et al. has helped advance the understanding of choroidal structure [20]. This imaging technique has made it possible to divide the choroid into different subregions by using binarization techniques on EDI-OCT images. Through these methods, the luminal (vascular) and stromal (interstitial) areas can be differentiated, and automated determination of quantitative values can be performed to identify vascularity changes.

Dogan et al. found that the mean SFCT value was significantly different between the MSON and MSNON groups (*p* = 0.004). However, they reported no significant differences in the MSON versus control and MSNON versus control comparisons [21]. Esen et al. reported there were no significant differences between the MSON and MSNON groups, in terms of mean SFCT values [22]. In the present study, although mean SFCT did not show a significant difference between the three groups, the CVI values were found to be significantly lower in the ON-affected eyes of MS patients compared to

their unaffected eyes and control eyes. It was also interesting to note that CVI values were also significantly lower in the unaffected eyes of MS patients when compared to the control group (*p* = 0.030). We thought that evaluating CVI values through the utilization of a semiautomated program which provides quantitative values may be more reliable than the assessment of SFCT in terms of assessing choroidal changes.

It should be noted that, until recently, MS was thought to be a myelin disorder; however, postmortem studies showed neuronal and axonal loss [5]. Gordon-Lipkin et al. suggested the presence of neuronal damage mechanisms other than demyelination in MS and reported a correlation between reduced RNFLT and brain atrophy, as measured by magnetic resonance imaging [23]. Besides, vascular disorganization mechanisms are known to be involved in the pathophysiology of MS. Recently, sensitive perfusion-weighted magnetic resonance imaging techniques have demonstrated decreased cerebral perfusion in patients with MS [24, 25]. It has been reported that there is an elevation in the plasma levels of endothelin-1, a potent vasoconstrictor peptide that could cause cerebral hypoperfusion if it was elevated in the spinal fluid. It has been
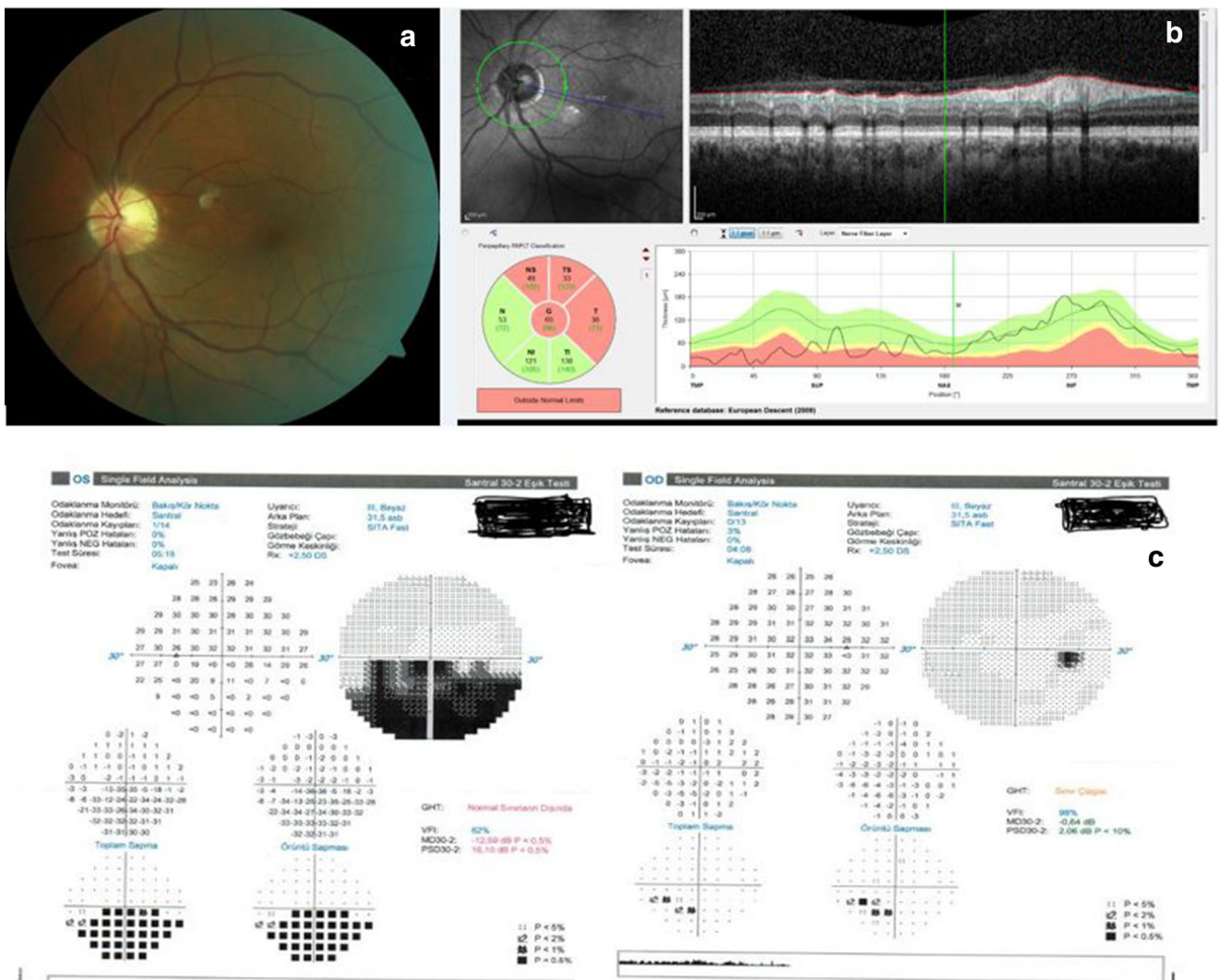
Fig. 2 Color fundus photo, OCT image of RNFLT map, and 24-2 SITA Humphrey visual field for the left affected eye (ON attack, 18 months prior to scan) in a patient with MS. **a** The fundus examination showed atrophic optic disc in the temporal, superior temporal, and superior nasal quadrants on the affected eye with ON attack of the MS patient. **b** The retinal nerve fiber layers were observed to be thinned in the temporal, superior temporal, and superior nasal quadrants on the same eye. **c** Showing the altitudinal inferior visual field loss

suggested that the elevation of endothelin-1 could contribute to neurodegeneration [26, 27]. Hypoperfusion causes axonal degeneration through disruption of mitochondrial oxygenation, decreased energy metabolism, and increased oxidative stress [28, 29]. Endothelin-1 is mainly secreted from reactive astrocytes [30]. Endothelin-1 not only acts as a potent vasoconstrictor but also augments the sensitivity of vessels to other vasoconstrictors, such as norepinephrine and angiotensin II [26]. As mentioned before, CVI was lower and total choroidal area and luminal area values were higher in patients with MS when compared to controls. Therefore, we hypothesized that such vascular disorganization in the MS could be disproportionately affecting the choroidal layer of the eye—especially the vascular area of the choroid rather than the stromal area. The significant anatomical changes seen, especially in the luminal area, may prove that vascular disorganization (which

is suggested to be involved in MS pathophysiology) may also be effective in the eye. Chen et al. documented thinner peripapillary choroid in patients with acute ON and suggested a relationship with endothelin-1 [31]. Also, decreased extraocular blood flow and increased endothelin-1 plasma levels have been reported in patients with MS [10]. Wang et al. studied the microcirculation of the optic nerve head in MS patients with and without ON history by using OCT angiography. They found that the mean optic nerve head flow index in the MSON group was significantly lower compared to healthy controls and the unaffected eyes of patients with MS. They hypothesized that ON attack could affect the perfusion of the vessel supplying the optic nerve. In the same study, no significant difference was reported between healthy control and MSNON groups in the optic nerve head flow index and parafoveal flow index among the three groups [32].

Interestingly, in the current study, the CVI, total choroidal area, and luminal area values that were studied in the subfoveal region of the choroid were observed to be significantly affected in MSNON eyes, as comparisons with the healthy subjects group demonstrated statistical significance ($p = 0.030$, $p = 0.031$, and $p = 0.013$, respectively). According to these results, choroidal vascular pathologies also appear to occur in eyes without ON history in patients with MS. Choroid thickness has been shown to be affected by many factors in past studies [33]. Although only patients with no history of cortisone use for at least 3 months were included in our study, it is possible that the systemic treatments used for MS (especially steroids) might affect these choroidal regions. As such, it is evident that we cannot reliably conclude that the changes demonstrated in this study are solely caused by the presence of MS and ON attacks; however, the differences shown in the comparison of the affected and unaffected eyes of MS patients provides evidence that this region is affected to a greater degree in the presence of ON history. We believe that these results strongly support the hypothesis that ON attacks have significant effects on vascular organization in the choroidal region.

Previous studies have shown the loss of retinal ganglion cells and axons in MS [34–37]. The presented study showed that, although retinal nerve fiber layer was thinnest in the MSON group, a significant difference was not observed when these three groups were compared ($p = 0.261$). Trip et al. reported decreased RNFLT in eyes with MSON compared to healthy eyes [38]. It was suggested to be because the eyes with MSNON were subclinical affected by retrograde transsynaptic degeneration during attacks, which might have caused chronic thinning in retinal nerve fiber layers [36, 39, 40]. However, there is no clear evidence to support this conclusion. Differences between studies may be related to differences in disease duration, age groups, use of corticosteroids and systemic drugs, and ON attack time (time surpassed since attack). Many studies have demonstrated that retinal nerve fiber layer loss is correlated with visual acuity, color vision, contrast sensitivity, multifocal evoked visual potentials, magnetic resonance imaging findings, and EDSS score [23, 41, 42].

Disease duration and EDSS scores are some of the parameters associated with progressive axonal loss in MS [43]. This study found a moderate negative correlation between CVI values and the EDSS score in the MSON group ($r = -0.407$ and $p = 0.002$). Furthermore, in the MSON group, it was determined that an increase of 1 unit in the EDSS score would cause a decrease of 7.074 units in CVI value [Beta (95% CI) = −7.074 (−12.867, −1.282), $p=0.020$]. According to these results, the degree of physical disability appears to be inversely correlated with CVI value, even though no significant reduction can be observed in RNFLT values.

This study has some limitations: the number of patients was limited and the mean duration with disease was not long

(3.12 ± 7.16 years). The study population was heterogeneous with regard to race and ethnic origin, which could alter results. Studies to be conducted with larger populations and longer follow-up may contribute to better evaluation of the choroid.

Conclusion

The current study demonstrated, to the best of our knowledge for the first time, CVI values were lower in the ON-affected and ON-unaffected eyes of patients with MS when compared to controls. Furthermore, the choroid was influenced to a greater degree in the affected eyes with ON attacks of MS patients, compared to their unaffected eyes. The significant anatomical changes (especially with regard to luminal area values) determined with EDI-OCT imaging may prove that vascular disorganization—which is argued to be involved in MS pathophysiology—also affects the eyes of patients with MS, especially in their affected eyes with ON. The CVI value, which is an indicator of the quantitative structural changes seen in the choroidal layer, might be also a useful prognostic factor in patients with MS.

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Declarations

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

Conflict of interest The authors declare no conflict of interest.

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