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Analysis of choroidal vascularity index in multiple sclerosis patients without optic neuritis attack

Emine Temel *, Nazife Aşıkgarip, Yusuf Koçak, Kemal Örnek, Özkan Kocamış, Gökçen Özcan

Department of Ophthalmology, Kırşehir Ahi Evran Training and Research Hospital, Turkey

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ABSTRACT

Background: To evaluate the choroidal structural changes in multiple sclerosis (MS) patients without previous optic neuritis (ON) attacks.

Methods: Forty eyes of 20 MS patients without a history of ON and 40 eyes of 20 age-matched healthy volunteers were included in this study. The choroidal thickness (CT) was measured at three points; subfoveal, 1500 μm nasal to the fovea, and 1500 μm temporal to the fovea. Choroidal area (CA), luminal area (LA), and choroidal vascularity index (CVI) were calculated using ImageJ.

Results: The mean subfoveal, nasal and temporal CT were decreased in MS patients compared to controls (for all, $p < 0.001$). The mean LA was 0.572 ± 0.113 mm^2 in MS group, and 0.729 ± 0.188 mm^2 in controls ($p = 0.002$). The mean CVI was decreased in the MS group ($69.38\% \pm 4.87$) in comparison to the controls ($73.41\% \pm 5.18$) ($p = 0.034$).

Conclusion: The current study demonstrated significant anatomical alterations of the choroid in the eyes of patients with MS.

1. Introduction

Multiple sclerosis (MS) is a chronic, autoimmune and demyelinating disease of the central nervous system [1]. It is one of the most common neurological disorders, affecting an estimated 2.3 million people worldwide [2]. The disease causes progressive neurodegeneration, often resulting in an increased burden of symptoms and impairments over time, including fatigue, spasticity, walking difficulty, dizziness, and sensory disturbances [3]. Optic neuritis (ON) is an inflammatory and demyelinating condition that causes acute visual loss. It is highly associated with MS [1].

Immune-mediated inflammatory demyelination has a significant role in the pathogenesis of MS [4]. Moreover, involvement of vascular factors has been suggested to cause vascular dysregulation during the course of the disease [5–7]. Thus, possible alterations in the hemodynamics of the orbital vessels have been of particular interest in MS patients and were assessed in several studies [5,6,8]. The results demonstrated a significant reduction in ocular blood flow parameters and impairment of retrobulbar hemodynamics, especially in the posterior ciliary arteries of the patients.

Being a highly vascularized tissue, the choroid may provide evidence supporting the potential role of vascular dysregulation in MS pathophysiology. It has been suggested that in vivo measurement of the subfoveal choroidal thickness (CT) can be an indirect indicator of local perfusion. Hence, it has become a parameter for the quantitative evaluation of the choroidal structure and has been used to show an association between the choroid and disease status [9,10]. In recent years, choroidal structural parameters and choroidal vascularity index (CVI) have been of great interest for researchers as new indicators of the choroidal vasculature.

Esen et al. measured CT in patients with MS using enhanced depth imaging optical coherence tomography (EDI-OCT) and found decreased CT compared with controls [11]. Garcia-Martin et al. found peripapillary choroidal thinning at all zones around the optic disk [12]. In the only study, Balcı et al. found decreased CVI in the affected and unaffected eyes of MS patients when compared with the healthy controls [13].

To the best of our knowledge, there is no research assessing the choroidal vasculature in MS patients without previous optic nerve involvement in both eyes. Therefore, we aimed to evaluate the choroidal structural changes and CVI in a group of MS patients without ON history.

* Corresponding author.
E-mail address: emine912@hotmail.com (E. Temel).

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2. Materıals and methods

Twenty patients with MS and 20 age-matched healthy volunteers were included in this prospective cross-sectional study. The study was approved by the Institutional Review Board Committee and was performed according to the ethical principles of the Declaration of Helsinki. Informed consent was obtained from all participants after explaining the nature of the study.

Exclusion criteria were as follows; eyes with BCVA less than 20/25 (Snellen), spherical equivalent refractive error more than ± 2.0 diopters and intraocular pressure (IOP) more than 21 mmHg, systemic (diabetes mellitus, hypertension, cardiovascular diseases) or local diseases (glaucoma, uveitis, high myopia, optic neuropathy, maculopathy), media opacities preventing adequate imaging, previous intraocular interventions (phacoemulsification or vitrectomy), history of smoking. None of the participants consumed caffeine and/or alcohol prior to the OCT examinations.

All patients were recruited from the outpatient clinic of the Neurology Department between June 2021 and September 2021. The diagnosis was based on the 2011 revision of the McDonald criteria [14]. The control group was randomly selected from the individuals who attended the Ophthalmology Department for routine examination.

The participants underwent a comprehensive ophthalmologic examination, including measurement of best-corrected visual acuity (BCVA), pupillary response and color vision (Ishihara), slit-lamp examination, fundoscopy, intraocular pressure (IOP) measurement and axial length (AL) measurements and enhanced depth imaging (EDI) OCT.

Enhanced depth imaging OCT (Spectralis®, SD-OCT; software version 6.3.3.0, Heidelberg Engineering Inc., Heidelberg, Germany) was performed on all participants after pupillary dilatation. All examinations were done within the same time interval (between 1:00 am and 3:00 pm) under dim light conditions. A single experienced technician captured the OCT images. EDI-OCT was conducted using the technique described by Spaide et al. [15]. Two examiners ET (operator 1) and NA (operator 2) performed all measurements in two different sessions. They were masked as to their previous measurements and to each other.

The CT was determined as the distance between the reflectivity border of the Bruch membrane and the sclerochoroidal interface. It was measured at three points; subfoveal, 1500 μm nasal to the fovea, and 1500 μm temporal to the fovea (Fig. 1).

The total choroidal area (TCA) was binarized to the luminal area (LA) and stromal area (SA) using ImageJ an open-code Java-based image processing software (version 1.50a; National Institutes of Health). The TCA was measured manually at 3000 micrometers wide, with margins of 1500 μm nasal and 1500 μm temporal from the foveal center horizontally and from the retina pigment epithelium (RPE) to the choroidoscleral border vertically. Binarization was performed using the Niblack auto local threshold method (Fig. 2). The white pixels were accepted as the SA, and the dark pixels were accepted as the LA [16]. The CVI, which was the proportion of the LA to the TCA, was calculated.

The analysis of the data was done in IBM SPSS 11.5 (SPSS Inc., Chicago, IL, USA) program. The Kolmogorov-Smirnow test was used to determine whether continuous variables were distributed normally. The Chi-squared test was used to compare between-gender data and the independent samples *t*-test to compare age between the groups. Agreement between intraobserver and interobserver measurements were assessed using the intraclass correlation coefficient. The correlation between the two eyes of the same subject was adjusted using generalized estimating equations during the calculation of summary descriptive parameters. Multivariate models adjusted using generalized estimating equations methods were fit to assess the effects of age, gender, SE and IOP on the CT and choroidal structural measurements. We used post hoc tests with Bonferroni correction. All values are given as mean ± standard deviation, and significance was considered at a p-value < 0.05.

3. Results

In total, there were 40 eyes of 20 patients with MS and 40 eyes of 20 healthy control subjects. The mean disease duration was 11.9 ± 2.2 years (range, 8–15). The demographic and clinical characteristics of the study participants are listed in Table 1.

On examination, there was no relative afferent pupillary defect, and color vision tests were normal in both groups. Dilated fundus examination revealed a clear media, normal peripheral retina, macula, and optic discs.

The mean subfoveal CT was 318.4 ± 41.4 μm (range, 212–398) in the MS group and 372.5 ± 45.4 μm (range, 296–409) in the control group.

The mean nasal CT was 258.4 ± 51.2 μm (range, 202–308) in MS group and 298.5 ± 59.4 μm (range, 216–354) in the control group. The mean temporal CT was 257.3 ± 42.3 μm (range, 201–306) in MS group and 309.4 ± 58.9 μm (range, 212–362) in the control group.

When compared, the mean subfoveal, nasal and temporal CT were significantly decreased in MS patients compared to healthy controls (for all, $p < 0.001$).

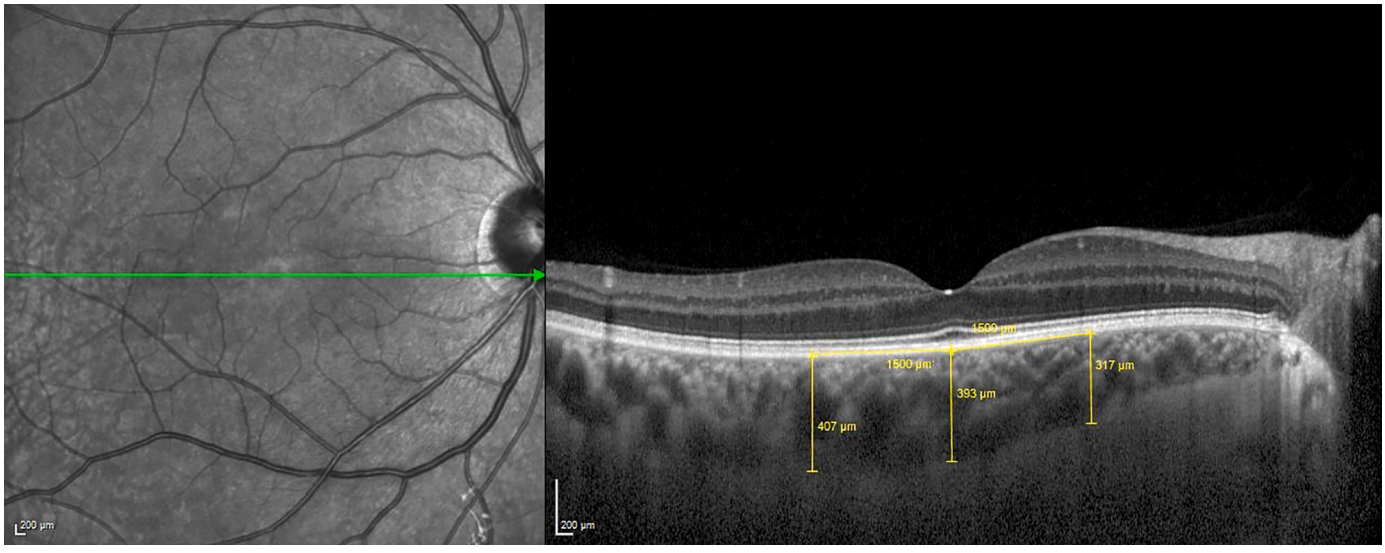

Fig. 1. Choroidal thickness was measured at three points; subfoveal, 1500 μm nasal to the fovea, and 1500 μm temporal to the fovea.

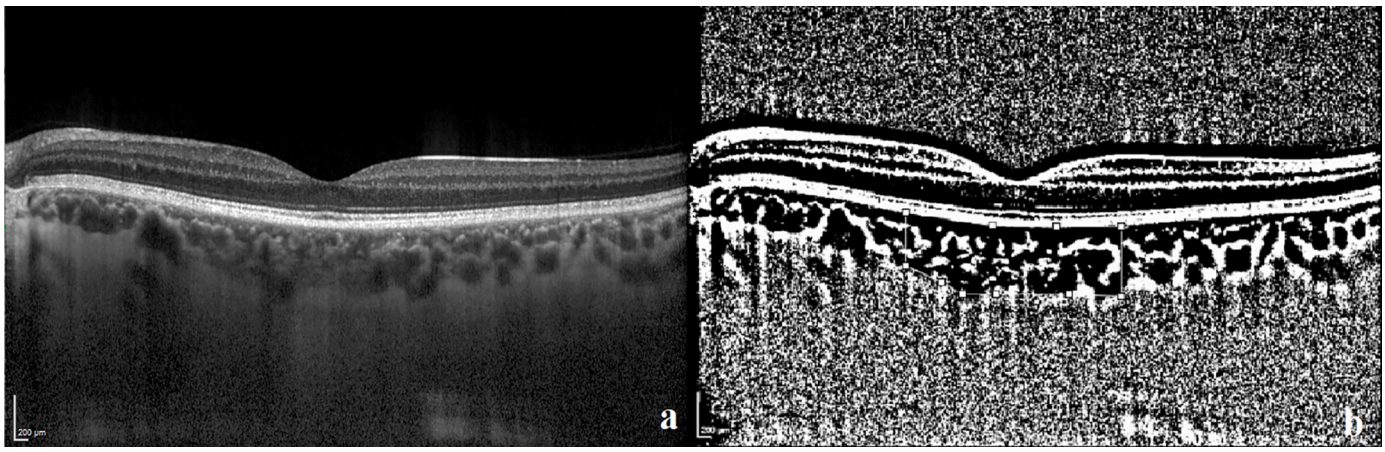

Fig. 2. Converted binary image using Image-J with the area of interest demarcated with a white line. The choroidal area was measured at approximately 3000 μm wide with the margins of 1500 μm nasal and 1500 μm temporal from the foveal center.

Table 1
The demographic and clinical characteristics of the study groups.

	Patients ($n = 20$)	Controls ($n = 20$)	p-value
Gender (n)			
Male / Female	8/12	8/12	0.526
Age (years)			
Mean ± SD	36.4 ± 4.6	36.5 ± 4.9	0.532
Range	(32–42)	(32–42)	
Mean BCVA (logMAR)	0.0	0.0	1.000
Intraocular pressure (mmHg)			
Mean ± SD	12.4 ± 1.05	12.2 ± 0.9	0.648
Range	(11–14)	(11–14)	
Refractive error (diopters)			
Mean ± SD	– 0.84 ± 0.52 D	– 0.84 ± 0.54 D	0.466
Range	(–1.0 to 0.75)	(– 1.0 to 0.75)	

SD: Standard deviation; BCVA: Best-corrected visual acuity.

The mean TCA was measured as 0.84 ± 0.10 mm^2 (range, 0.61–1.03) and 0.98 ± 0.23 mm^2 (range, 0.61–1.19) ($p = 0.013$), the mean LA was 0.57 ± 0.11 mm^2 (range, 0.31–0.83), and 0.72 ± 0.18 mm^2 (range, 0.43–1.17) ($p = 0.002$), the mean SA was 0.25 ± 0.06 mm^2 (range, 0.12–0.38) and 0.25 ± 0.06 mm^2 (range, 0.16–0.40) ($p = 0.342$) for the MS group and the controls, respectively.

The mean CVI was significantly decreased in the MS group [69.38% ± 4.87 (range, 53.42–74.69)] in comparison to the control group [73.41% ± 5.18 (range, 66.79–79.64)] ($p = 0.034$).

We found that age was the only significant variable correlating with CT at all locations. Age had a negative correlation with the CT ($p = 0.012$, $r = -0.12$).

There was a statistically significant strong association between the LA, CVI and SE of the participants ($r = 0.741$, $p = 0.001$, and $r = -0.619$; $p = 0.001$) (Figs. 2, 3). Age and the AL of the participants were not associated with the LA and the CVI. ($r = -0.089$, $p = 0.262$, $r = 0.019$, $p = 0.812$, and $r = 0{,}062$, $p = 0.481$ and $r = -0.138$, $p = 0.079$, respectively).

A multiple regression analysis was performed to determine the effects of the gender, CVI, TCA, and SE on the LA. We found that the TCA and SE had a significant effect on the LA.

The intraobserver and interobserver differences are summarized in Table 2. There were no significant differences between the measurements of the two examiners.

Table 2
Repeatability and reproducibility of the measurements.

	Cronbach's Alpha coefficient	Intraclass correlation coefficient	95% CI	
			Lower	Upper
TCA				
Repeatability Operator 1	0.997	0.997	0.996	0.998
Repeatability Operator 2	0.998	0.998	0.997	0.999
Reproducibility Operator 1 versus Operator 2	0.996	0.996	0.995	0.997
LA				
Repeatability Operator 1	0.996	0.996	0.995	0.997
Repeatability Operator 2	0.997	0.997	0.995	0.998
Reproducibility Operator 1 versus Operator 2	0.994	9.994	0.990	0.996
SA				
Repeatability Operator 1	0.997	0.997	0.996	0.998
Repeatability Operator 2	0.997	0.997	0.995	0.998
Reproducibility Operator 1 versus Operator 2	0.995	9.995	0.992	0.997
CVI				
Repeatability Operator 1	0.996	0.996	0.995	0.997
Repeatability Operator 2	0.997	0.997	0.995	0.998
Reproducibility Operator 1 versus Operator 2	0.988	0.988	0.981	0.992

TCA: Total choroidal area; LA: Luminal area; SA: Stromal area; CVI: Choroidal vascularity index.

4. Discussion

According to the results of the current study, the subfoveal, nasal and temporal CT, as well as the structural parameters and CVI were significantly decreased in MS patients without previous ON when compared with the healthy control group.

Ocular blood flow studies were conducted in MS patients to assess the changes in ocular hemodynamics, using color Doppler imaging technique. Pache et al. demonstrated a significant reduction in peak systolic and end-diastolic velocities of the ophthalmic, posterior ciliary and central retinal arteries [17]. Plasma endothelin-1, a potent vasoconstrictor, levels were significantly increased in the patients [17]. Modrzejewska et al. showed blood flow velocity disturbances in the

short posterior ciliary and central retinal arteries of the previously affected (ON) and unaffected fellow eyes in MS patients [18]. All these results support a possible association of impaired retrobulbar hemodynamics with MS.

Enhanced depth imaging OCT is a new technique that has allowed the ophthalmologists to learn more about the choroid since its first release several years ago [15]. Thus, it has been possible to separate the choroid into different subregions using the binarization method on EDI-OCT images. Through these, the luminal and stromal areas of the choroid can be differentiated, and automated determination of quantitative values could be done to identify the vascular changes.

Dogan et al. evaluated CT changes in 104 eyes of 52 MS patients (35 females, 17 males) with and without ON using EDI-OCT [19]. The disease duration was 6.37 ± 5.3 years. In their study, there were no significant differences for the subfoveal, nasal, and temporal CT in MS patients in comparison to the healthy controls. They found significantly decreased CT at all segments in patients without ON (62 eyes) compared with the ON group (42 eyes) [19]. Esen et al. measured CT in 68 eyes of 34 MS patients (31 relapsing-remitting, 3 progressive) using EDI-OCT. There were 24 female and 10 male patients. The disease duration was 7.3 ± 4.8 years. At all quadrants, the CT was significantly decreased in MS patients with or without ON with respect to the healthy controls [11]. The authors suggested that decreased CT may be the result of impaired blood perfusion due to the inflammatory response. Contrary to the results of Dogan et al., Esen et al. did not find any significant differences in CT between MS patients with a previous ON and those without ON [11]. Also, there was no association between the CT and disease duration. In our study, the mean disease duration was 11.9 ± 2.2 years. At all segments, CT was significantly decreased in MS patients without previous ON in comparison to the healthy control group.

The disparities between the results of the studies can be due to the history of ON, gender differences, or duration of the disease. Many studies have demonstrated the presence of hypoxia and hypoperfusion in the central nervous system of MS patients [20,21]. Hypoperfusion can be observed from the very early course of the disease in patients with MS. There may be a reduction in cerebral blood flow in affected individuals, even in the absence of structural damage [22,23]. Preliminary data suggest that retinal blood flow is reduced in patients with relapsing-remitting MS compared with healthy controls [24,25]. In two different OCT angiography studies including advanced MS cases, vessel density around the optic nerve head was found to be decreased in MS eyes compared with control eyes, as well as in MS with ON compared with MS without ON [26,27]. All these data show that the onset and severity of MS or the presence of ON involvement can affect the microvascular status of the choroid. Multiple sclerosis is more prevalent in females than males. As known, female and male genders can have different genetic, nervous system and immune system responses. The effect of gender on clinical features of MS is not as clear as the effect on prevalence; however, there is evidence that females have an early onset of disease, they have a greater tendency to develop MS and male patients can have a worse prognosis with MS [28].

When the literature was searched, there is only one study evaluating the choroidal structural parameters in MS patients. Balcı et al. investigated the CVI changes in MS patients using binarized EDI-OCT images and evaluated the effect of ON on these parameters [13]. They found that the CVI was decreased in the unaffected eyes of patients who had a history of ON. In the study, the CVI was significantly decreased in the eyes of the MS patients without previous ON in comparison to the healthy control group. Additionally, in accordance with the results of our study, the TCA and LA were also significantly decreased in MS patients without previous ON attack.

The choroid supplies blood flow to the retinal pigmented epithelium, photoreceptors, and the prelaminar portion of the optic nerve [30]. This densely vascularized tissue can be affected by systemic inflammatory and vascular diseases [31]. Agrawal et al. assessed CVI in patients with posterior uveitis and panuveitis and they found an increased CVI in the affected eyes, which was significantly decreased after 3 months [32]. Significant choroidal changes as decreased CVI were also reported in eyes with serpiginous choroiditis associated with tuberculosis [33]. Liu et al. demonstrated decreased CVI during the active phase of the Vogt-Koyanagi-Harada disease and hypothesized that the decrease was due to choroidal stromal edema and inflammatory cell infiltration [34].

Chronic inflammation seems to play a major role in the initiation of neurodegenerative process in different forms of MS. The disease is characterized by increased inflammatory mediators in the vascular system. Endothelial dysfunction, as well as platelet activation and thrombophilia, have been shown in MS [35,36]. Previous studies have demonstrated decreased cerebral and ocular perfusion in patients with MS [22–27,37]. We assume that vascular changes like impairment of the blood flow in the posterior ciliary arteries, hypercoagulability, and hyper-inflammation factors, which have been shown to be involved in the pathogenesis of MS, may partly or fully contribute to the effect of MS on the choroidal vascular parameters of the patients.

Although CT has been widely used in clinical trials, it reflects only the total choroidal structure without any distinction about the inner morphology of the choroidal vasculature. In healthy eyes from a population-based study, Agrawal et al. compared the factors affecting subfoveal CT to those that affect CVI, and they found that there were significant associations between the subfoveal CT and several factors like age, AL, intraocular pressure and, most significantly, the LA of the choroid [29]. In our study, we found significantly decreased CT and CVI in patients with MS. There was a significant difference between the two groups for the luminal area. There was a statistically significant association between the LA, CVI and SE of the participants. We found that the TCA and SE had a significant effect on the LA.

This study has some limitations; it has a cross-sectional design, therefore longitudinal studies should investigate the effect of MS on the choroidal structure further. Second, the sample size is relatively small. Studies to be conducted with larger populations and longer follow-up may contribute to a better assessment of the choroidal structures and their association with the disease progression. The strength of our study is the comparison of the choroidal structural parameters and the CVI in a group of MS patients without previous ON attack in both eyes.

In conclusion, the results showed significant choroidal structural changes in MS patients without previous ON attack. The CVI, which is an indicator of the quantitative structural changes in the choroid, may complete the existing tools in the diagnosis or progression of the MS patients with or without previous ON. Larger trials are still needed to assess the choroidal structural and vascular changes in patients with MS and to lay down how this technique can be used in the clinical setting to understand the underlying pathogenesis more clearly.

CRediT authorship contribution statement

Emine Temel: Formal analysis, Methodology, Investigation, Conceptualization, Data curation, Writing – review & editing. **Nazife Aşıkgarip:** Methodology, Formal analysis, Data curation, Conceptualization. **Yusuf Koçak:** Conceptualization, Data curation, Formal analysis. **Kemal Örnek:** Formal analysis, Data curation, Conceptualization. **Özkan Kocamış:** Conceptualization, Data curation, Formal analysis. **Gökçen Özcan:** Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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