TITLE PAGE

Title: ENHANCED DEPTH IMAGING OPTICAL COHERENCE TOMOGRAPHY OF THE CHOROID IN CENTRAL RETINAL VEIN OCCLUSION

Short title: Choroidal Thickness in Central Retinal Vein Occlusion

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Abstract (MUST be submitted as a separate file)

PURPOSE: To evaluate subfoveal choroidal thickness in patients with central retinal vein occlusion (CRVO) using enhanced depth imaging (EDI) optical coherence tomography (OCT).

DESIGN: Retrospective observational study.

METHODS: We measured bilateral subfoveal choroidal thickness, averaged for 100 scans, in 36 patients (mean age, 66 ± 15 years; 26 women and 10 men) with unilateral CRVO by using the EDI modes of the Spectralis OCT system. Twenty-two patients were treated with intravitreal bevacizumab (1.25 mg/0.05 mL), and subfoveal choroidal thickness was measured before and after treatment. Statistical analysis was performed to compare subfoveal choroidal thickness of CRVO and fellow eyes, and subfoveal choroidal thickness before and after intravitreal bevacizumab.

RESULTS: Mean subfoveal choroidal thickness measured in 36 eligible eyes of 36 patients was 257.1 ± 83.2 μm, which was significantly greater than that in fellow eyes (222.6 ± 67.8 μm) ($P < .01$, paired t-test). There was strong correlation between CRVO eyes and fellow eyes ($r = .79$, $P < .01$). Mean subfoveal choroidal thickness after intravitreal bevacizumab was 227.7 ± 65.1 μm, which was significantly thinner than that before intravitreal bevacizumab

therapy (266.9 ± 79.0 μm) ($P < .01$, paired t-test).

CONCLUSIONS: Subfoveal choroidal thickness of CRVO eyes was significantly greater than that of fellow eyes and significantly decreased after intravitreal bevacizumab treatment. EDI-OCT can be used to evaluate choroidal involvement in CRVO and may assist noninvasive diagnosis and management of this disease.

INTRODUCTION

Central retinal vein occlusion (CRVO) is a common cause of unilateral visual loss, and macular edema is a frequent cause of vision loss. Although it is well known that central macular thickness increases because of severe macular edema in CRVO, we could find no report on subfoveal choroidal thickness in patients with CRVO. Spaide and associates used spectral-domain optical coherence tomography (OCT) and developed a method termed enhanced depth imaging (EDI) OCT that enables in vivo cross-sectional imaging of the choroid and measurement of the thickness of the choroid.[1] Subsequently, the subfoveal choroidal thickness of patients with various diseases, such as central serous chorioretinopathy,[2-4] macular hole,[5] age-related macular degeneration,[6-9] high myopia,[10,11] and Vogt-Koyanagi-Harada disease,[12] was reported. Further, certain reports noted changes in subfoveal choroidal thickness after treatment.[4,7,11-14] Because macular edema in patients with CRVO appears to be closely related to vascular endothelial growth factor (VEGF) levels in the vitreous,[15,16] inhibiting VEGF appears to be a reasonable therapeutic approach.[17-19] Although many studies have reported that central macular thickness in CRVO decreases after anti-VEGF treatment, such as intravitreal bevacizumab therapy, we could find no reports of changes in choroidal thickness in CRVO patients after treatment. The aim of this study was to compare the choroidal thickness in the macular area between eyes with CRVO and unaffected fellow eyes and to investigate how subfoveal choroidal thickness changes after intravitreal bevacizumab.

METHODS

A retrospective analysis was performed for consecutive patients examined with unilateral CRVO at our retinal outpatient department in the Department of Ophthalmology of the University of Nagasaki from July 2010 through May 2012. The clinical examination for diagnosis of CRVO included measurement of best-corrected visual acuity (BCVA), slit-lamp biomicroscopy with a contact lens or noncontact lens, indirect ophthalmoscopy, digital fluorescein angiography

(FA), and indocyanine green angiography (ICGA) (Heidelberg Retinal Angiography, Heidelberg, Germany). We obtained BCVA measurements for analysis by using a Japanese standard decimal visual chart and the logarithm of the minimum angle of resolution (logMAR) scale. Patients with macular edema were treated with intravitreal bevacizumab. The intravitreal bevacizumab treatment followed the tenets of the Declaration of Helsinki and approval was obtained from the Ethics Committee of Nagasaki University School of Medicine. Subfoveal choroidal thickness was measured using the Heidelberg Spectralis (Heidelberg Engineering, Heidelberg, Germany) according to the EDI-OCT technique described by Spaide. The camera was positioned close enough to the eye to obtain an inverted image of the choroid. This image was averaged over 100 scans with the automatic averaging and eye tracking features. The horizontal section going directly through the center of the fovea was selected. The resulting images were viewed and measured using the Heidelberg Eye Explorer software (version 1.6.40; Heidelberg Engineering). The choroid was measured from the outer portion of the hyper-reflective line corresponding to the retinal pigment epithelium (RPE) to the inner surface of the sclera. These measurements were obtained at the subfoveal choroid. Each image was measured by 3 independent observers (Y.M., R.U., and E.T.), with discrepancies of more than 20% being resolved by open adjudication with the senior author (E.T.). The visual acuities are stated as decimal equivalents and logMAR equivalents. The data obtained were analyzed with descriptive statistics. Measurements of choroidal thickness and retinal thickness were analyzed using Student *t*-test. Spearman's rank correlation coefficient was used to evaluate the correlation between choroidal thickness of the CRVO eyes and that of the fellow eyes. For all tests, $P < .05$ was considered significant.

RESULTS

There were 36 patients with unilateral CRVO; their mean age was 66 ± 15 years (range, 26-84 years). Twenty-six patients (72%) were women. The type of CRVO was ischemic in 10 (28%) patients and non-ischemic in 26(72%). These

patients had additional systemic diseases: 23 (64%) had hypertension and 8 (22%) had diabetes mellitus without retinopathy. The full -thickness of the choroid could be visualized in all 36 eyes. BCVA (logMAR) before treatment ranged from -0.08 to 2.0 (median, 0.40). Twenty-two (61%) patients were treated with intravitreal bevacizumab to decrease macular edema, and subfoveal choroidal thickness was measured 1 month after intravitreal bevacizumab. The baseline demographic and clinical characteristics of the CRVO and fellow eyes are reported in Table 1. A negative correlation was found between subfoveal choroidal thickness and axial length in the CRVO eyes ($r = .28$, $P < .05$). Compared with fellow-eyes, the CRVO eyes showed significantly greater subfoveal choroidal thickness ($P < .01$, paired *t*- test). The mean ± SD subfoveal choroidal thickness was 257.1 ± 83.2 μm in the CRVO eyes and 222.6 ± 67.8 μm in the fellow eyes (Figure 1). A strong correlation was found between subfoveal choroidal thickness in the CRVO eyes and that in the fellow eyes (r = .79, P < .01). The mean subfoveal choroidal thickness was 252.6 μm (ischemic) and 258.8 μm (non-ischemic) in the CRVO eyes and 217.6 μm (ischemic) and 224.5 μm (non-ischemic) in the fellow eyes. Similarly CRVO eyes showed significantly greater subfoveal choroidal thickness ($P < .01$, paired *t*- test) in both the ischemic and non-ischemic groups. There was no difference between the ischemic and non-ischemic groups. Figure 2 shows EDI- OCT images of the choroid of 1 eye with CRVO and that of the unaffected fellow eye. For CRVO eyes, subfoveal choroidal thickness was significantly greater before intravitreal bevacizumab therapy than after therapy (P < .01, paired *t*- test). The mean ± SD subfoveal choroidal thickness in the CRVO eyes was 266.9 ± 79.0 μm before intravitreal bevacizumab and 227.7 ± 65.1 μm after intravitreal bevacizumab (Figure 3). Similarly, there was no difference between the ischemic and non-ischemic groups, and subfoveal choroidal thickness after intravitreal bevacizumab significantly decreased in both the ischemic (210.3 from 251.5μm) and non-ischemic (237.6 from 275.7 μm) groups (P < .05, paired *t*- test). Figure 4 shows EDI- OCT images of the choroid of 1 eye with CRVO before and after intravitreal bevacizumab treatment.

DISCUSSION

In this study, EDI-OCT measurements of subfoveal choroidal thickness demonstrated that the choroid of CRVO eyes is significantly greater than that of fellow eyes. Furthermore, we found that subfoveal choroidal thickness decreased after treatment with bevacizumab. EDI-OCT using the Heidelberg Spectralis is a common technique that allows direct in vivo measurement of choroidal thickness. Many studies have reported measurements of subfoveal choroidal thickness in various diseases.[2-14] Reibaldi et al[5] reported that choroidal thickness was reduced in eyes with idiopathic macular hole and also in fellow unaffected eyes. Imamura et al[2] and Maruko et al[3,12] reported that the choroid was markedly thick in patients with central serous chorioretinopathy and Vogt-Koyanagi-Harada disease, and that choroidal thickness decreased after treatment. Using high-penetration OCT, Ikuno and associates[20] found a mean subfoveal choroidal thickness of 354 μm (range, 80-641 μm) in healthy Japanese subjects, with a significant negative correlation with axial length and age. They estimated a decrease of 14 μm for each decade of life. Although mean subfoveal choroidal thickness of the CRVO and fellow eyes (257.1 μm and 222.6 μm, respectively) in our study was less than that in the previous study,[20] this difference between the studies may have been caused by differences in the measurement software, the OCT light source, the patient profile (e.g., age). Maruko et al[3,4] performed ICGA and found that subfoveal choroidal thickness in the fellow eyes of patients with central serous chorioretinopathy was increased in the eyes with choroidal vascular hyperpermeability. They suggested that hyperpermeability causes choroidal thickening through accumulation of fluid and that dilation of choroidal vessels plays a partial role in the choroidal thickening. In our study, we performed ICGA in certain cases; however, choroidal hyperpermeability was not clearly demonstrated. Therefore, increased subfoveal choroidal thickness may be related to not only choroidal hyperpermeability but also another cause. Choroidal blood flow is the highest of any tissue in the body to satisfy the normal metabolic

demands of the outer retina.[21] CRVO patients may be experiencing retinal hypoxia. Because of tissue hypoxia, VEGF expression increases in RPE, pericytes, and microvascular endothelial cells.[22] VEGF induces vessel dilation and increased ocular blood flow through a mechanism involving increased nitric oxide production,[23,24] and it increases vascular permeability in the eye.[25] Therefore, we speculated that vessel dilation and increased permeability caused by VEGF were related to increased subfoveal choroidal thickness in our study. Ellabban et al[13] reported that subfoveal choroidal thickness remains unchanged in eyes with neovascular age-related macular degeneration after intravitreal ranibizumab. In contrast, Yamazaki et al[14] reported that subfoveal choroidal thickness decreased after intravitreal ranibizumab in eyes with neovascular age-related macular degeneration. Although it might be difficult to detect minimal changes in thickness because of high variance in measurements of choroidal thickness, subfoveal choroidal thickness was significantly reduced after intravitreal bevacizumab in the present study. Because VEGF has various pharmacologic actions on the choroid,[23-25] inhibition of VEGF by bevacizumab may cause decreased subfoveal choroidal thickness in CRVO patients.

This retrospective study had several limitations, including a small sample size and short-term follow-up. Furthermore, there might have been other factors, yet to be investigated, that affected subfoveal choroidal thickness. Further study is required with a larger number of patients than that in the present study because of known interactions between choroidal thickness and age as well as refractive error. Increased subject numbers and longer follow-ups might demonstrate the relationship between choroidal thickness and recurrence. In addition, subfoveal choroidal thickness measurements were manually obtained in a retrospective manner; automated software is required for a more objective evaluation. We are currently performing longitudinal studies on changes in choroidal thickness after treatment, as well as possible relationships with clinical manifestations and visual prognosis.

In conclusion, subfoveal choroidal thickness of the CRVO eye is significantly greater than that of the fellow eye and is decreased after intravitreal

bevacizumab. Subfoveal choroidal thickness can be used to assess the effects of choroidal vascular changes by measuring choroidal thickness noninvasively with EDI-OCT. These findings may help to elucidate the pathophysiologic features of CRVO as well as its response to treatment. The possible role of the choroid in CRVO development needs to be investigated further.

Acknowledgement

All authors have completed and submitted the ICMJE form for disclosure of potential conflicts of interest, and none were reported. Contributions of authors: design of the study (E.T., K.S.), conduct of the study (K.S., T.K.), analysis and interpretation of data (E.T., U.R., Y.M., K.S.), writing the article (E.T.), critical revision of the article (E.T.), data collection (E.T., R.U., Y.M.), literature search (E.T., U.R.) and preparation (E.T.), and final approval of the manuscript (T.K.).

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FIGURE CAPTION

Figure.1 The comparison of subfoveal choroidal thickness between in the central retinal vein occlusion (CRVO) eyes and in the fellow eyes. Subfoveal choroidal thickness is significantly greater in eyes with CRVO (257.1 μm, SD= 83.2) than in the fellow eyes (222.6 μm, SD= 67.8)

Figure.2 Subfoveal choroidal thickness in patients with central retinal vein occlusion (CRVO) using enhanced depth imaging (EDI) optical coherence tomography (OCT). EDI-OCT performed vertically through the center of the fovea shows the cross-sectional choroidal structure (between retinal pigment epithelium line and arrowheads). Subfoveal choroidal thickness was (Top) 383 um in the right eye with CRVO of 67-year-old woman, (Bottom) 289 um in the fellow eye.

Figure.3 The comparison of subfoveal choroidal thickness between before and after intravitreal bevacizumab treatment in the central retinal vein occlusion eyes. Subfoveal choroidal thickness is significantly thinner after treatment (227.7 μm, SD= 65.1) than before treatment (266.9 μm, SD=79.0)

Figure.4 Subfoveal choroidal thickness in patients with central retinal vein occlusion (CRVO) using enhanced depth imaging optical coherence tomography before and after intravitreal bevacizumab treatment. Subfoveal choroidal thickness was (Top) 294 um in the right eye with CRVO of 84-year-old woman before treatment, (Bottom) 204 um after treatment.

Table

TABLE. Demographics and Clinical Characteristics of Central Retinal Vein Occlusion, Fellow Eye, and Intravitreal Bevacizumab Groups

	CRVO Group (n = 36)	Fellow eye Group (n = 36)	Intravitreal bevacizumab Group (n = 22)	*P* Value
Mean age +/- SD (yrs)	66 +/- 15	66 +/- 15	66 +/- 14	NS
Gender (female/male)	26 / 10	26 / 10	16 / 6	NS
Mean +/- SD axial length (mm)	23.2 +/- 1.3	23.3 +/- 1.2	23.1 +/- 1.4	NS
Type (ischemic / non-ischemic)	10 / 26	10 / 26	8 / 14	NS
Systemic disease				
Hypertension (+ /-)	23 / 13	23 / 13	13 / 9	NS
Diabetes Mellitus (+ /-)	8 / 28	8 / 28	7 / 15	NS

CRVO = central retinal vein occlusion; NS = not statistically significant; SD = standard deviation; yrs = years.

Figure1

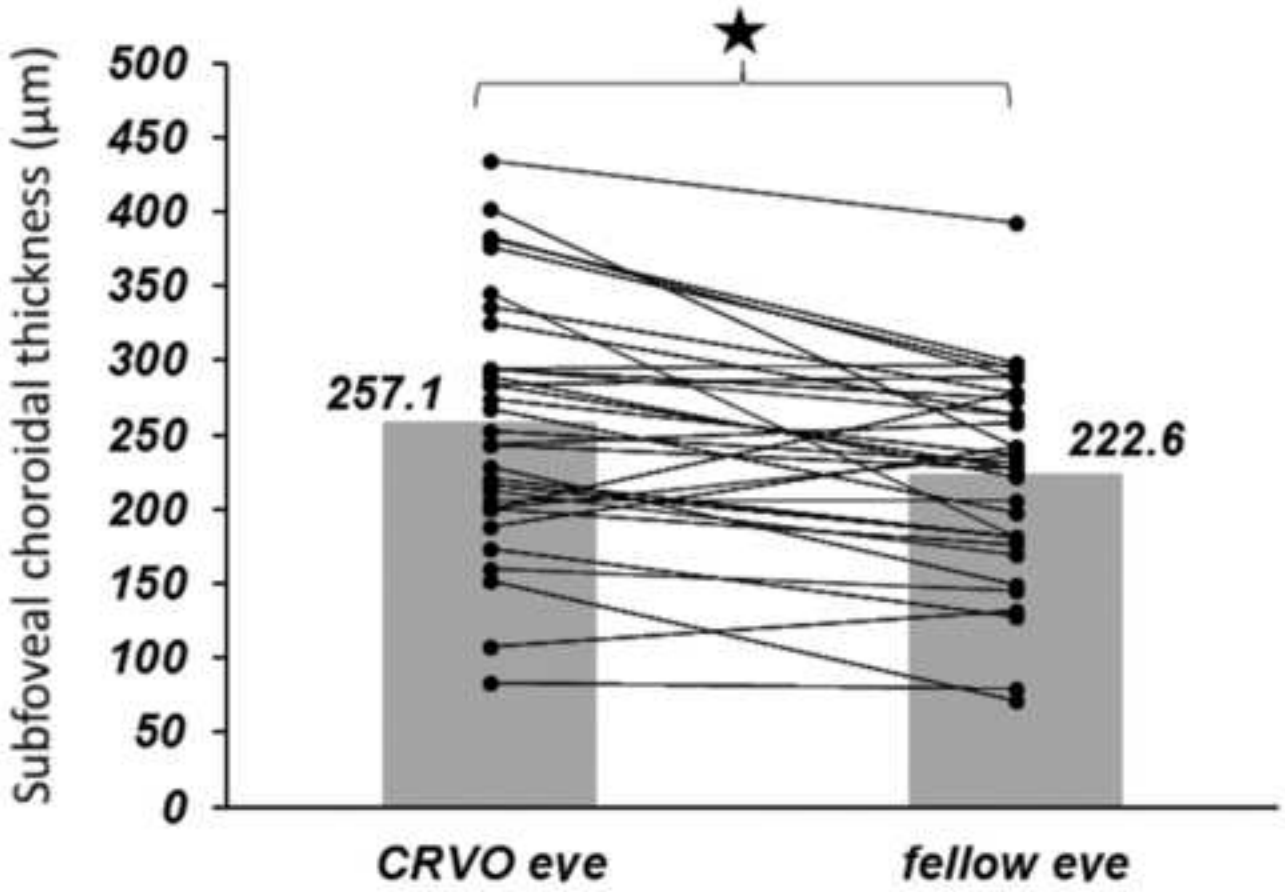
Subfoveal choroidal thickness (μm)
500
450
400
350
300
250
200
150
100
50
0
257.1
222.6
CRVO eve
fellow eve

Figure 2

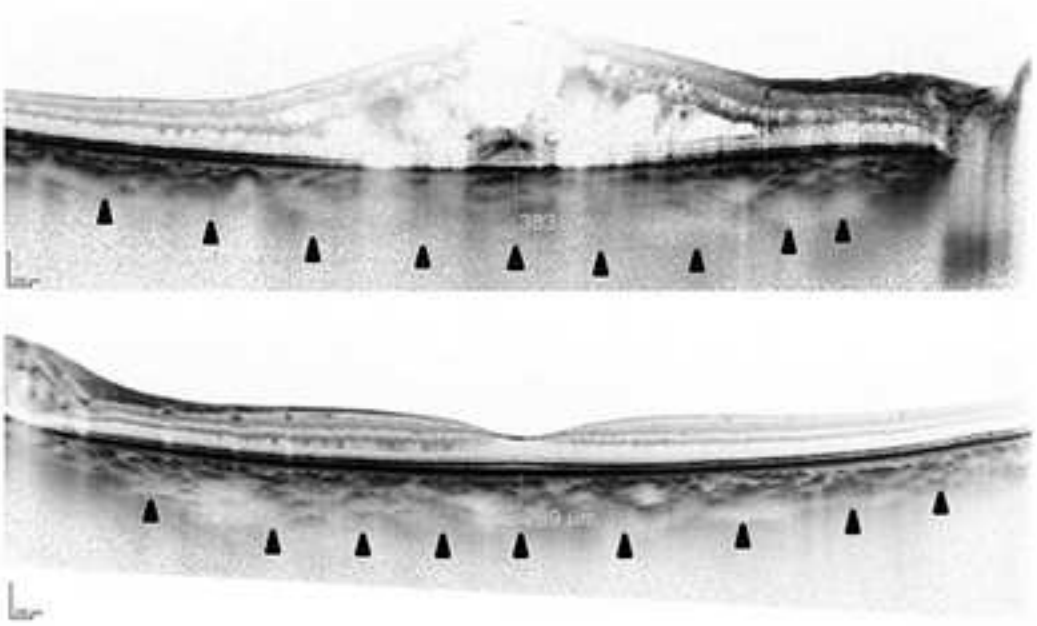

Figure (3)

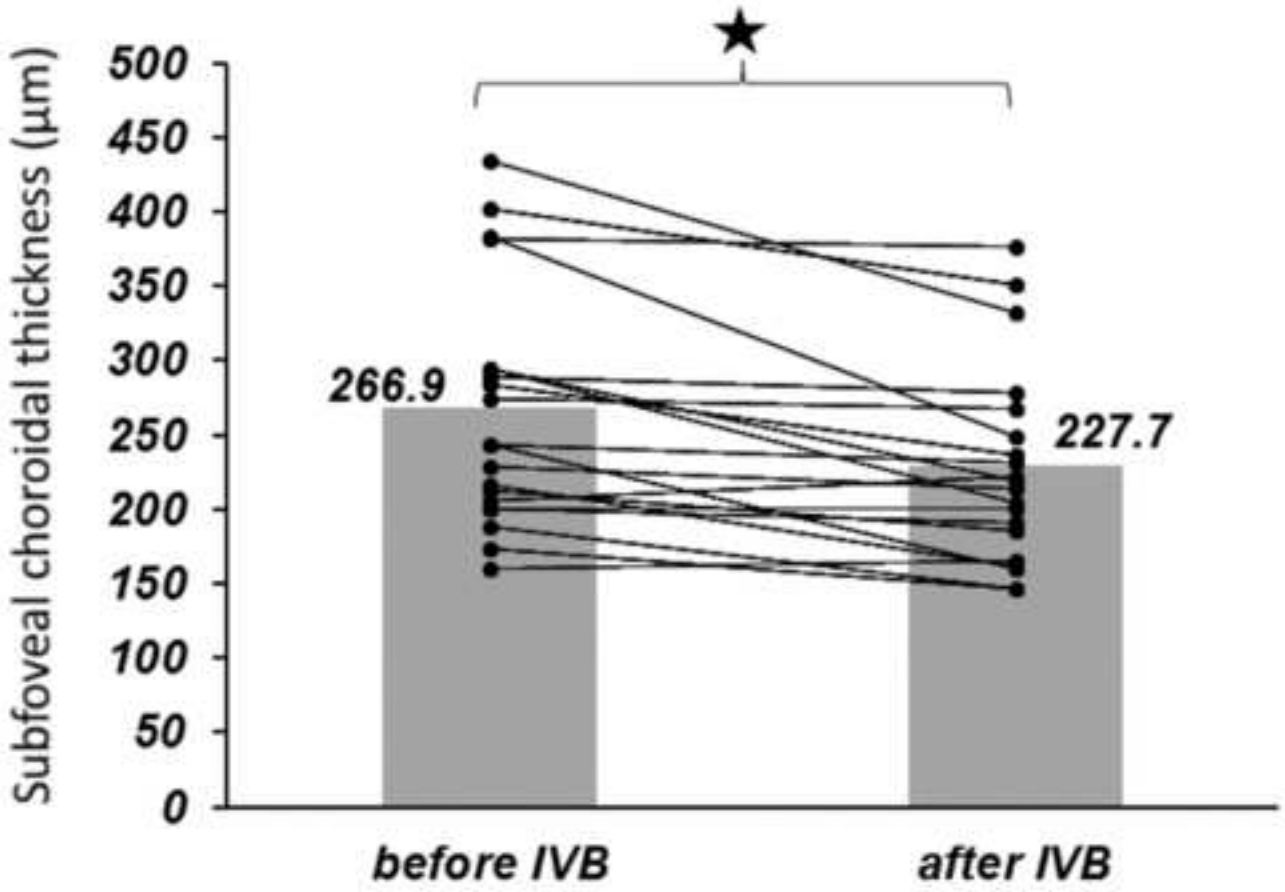
Subfoveal choroidal thickness (µm)
500
450
400
350
300
250
200
150
100
50
0
266.9
227.7
before IVB
after IVB

Figure 4

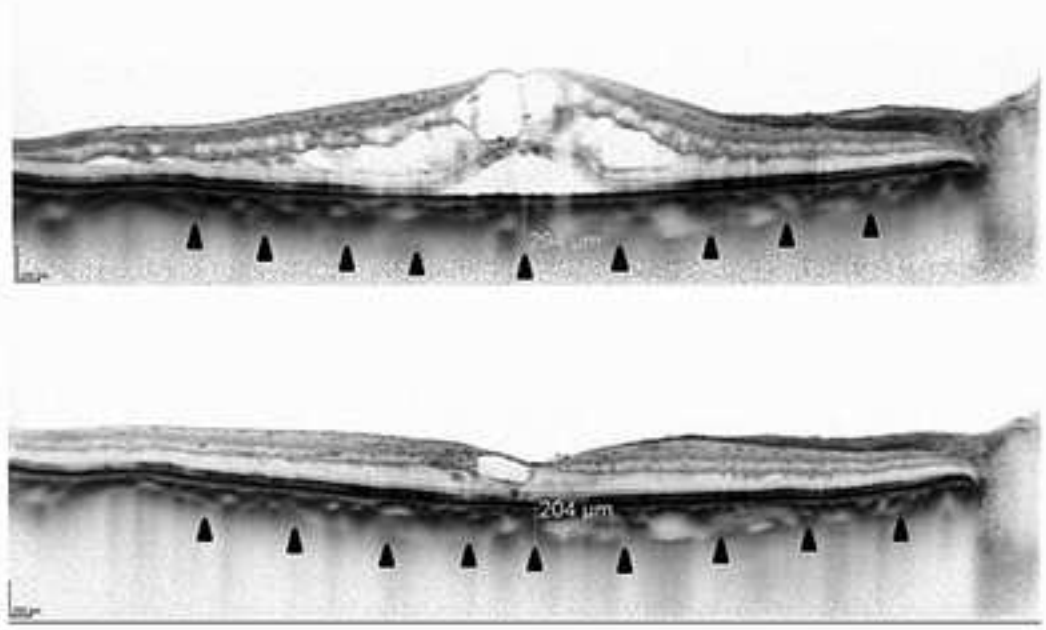

Table of Contents Statement

Subfoveal choroidal thickness was measured in patients with central retinal vein occlusion (CRVO) by using the enhanced depth imaging (EDI) optical coherence tomography (OCT). Mean subfoveal choroidal thickness of CRVO eyes was significantly greater than that of fellow eyes and significantly decreased after intravitreal bevacizumab treatment. EDI-OCT can be used to evaluate choroidal involvement in CRVO and may assist noninvasive diagnosis and management of this disease.