Choroidal vascularity index in thyroid-associated ophthalmopathy: a cross-sectional study

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Abstract

Background: Hemodynamic changes have been observed in patients with Graves’ disease. The aim of our study was to evaluate choroidal vascular change using the choroidal vascularity index (CVI) in patients with thyroid-associated ophthalmopathy (TAO).

Methods: In this cross-sectional observational study, 40 patients affected by TAO were recruited. Forty healthy individuals, matched for age and sex, served as controls. Foveal enhanced-depth imaging optical coherence tomography scans were obtained from all participants. Images were binarized using the ImageJ software and luminal area (LA) and total choroidal area (TCA) were measured. CVI was calculated as the proportion of LA to TCA. The relation between CVI or subfoveal choroidal thickness (SFCT) and clinical activity score, exophthalmometric value, diplopia status, gender, and age was evaluated.

Results: CVI was significantly higher in patients with TAO (P = 0.004). No significant difference was observed in SFCT (P = 0.200) and TCA (P = 0.153) comparing TAO patients and healthy controls. LA was significantly higher in TAO group (P = 0.045). On multiple regression analysis, CVI was associated with TCA (P = 0.043). No association was found between SFCT or CVI and TCA, clinical activity score, exophthalmometric value, Inami value, diplopia status, gender or age (P > 0.05).

Conclusions: This is the first study that has demonstrated an increase in CVI in eyes with TAO compared with healthy controls and has assessed its association with clinical features.

Keywords: Choroidal vascularity index, Choroidal vasculature, Enhanced depth optical coherence tomography, Image binarization, Luminal area, Subfoveal choroidal thickness, Thyroid-associate ophthalmopathy

Background

Thyroid-associated ophthalmopathy (TAO), also called Graves’ ophthalmopathy or Graves’ orbitopathy, is an autoimmune disorder involving the orbital tissue, commonly found in patients with Graves’ disease [1]. Symptomatology ranges from ocular irritation and dryness in mild forms to redness, chemosis, edema and erythema of eyelids and diplopia [1]. Potential sight-threatening conditions such as corneal ulceration and compressive optic neuropathy may manifest in the most severe cases [2]. The pathogenesis of TAO has not been completely elucidated and is considered as the result of a combination of genetic and environmental factors [3]. Several risk factors have been investigated, including tobacco smoking and number of cigarettes smoked per day, older age at diagnosis of Graves’ hyperthyroidism, longer...
duration of the disease, uncontrolled thyroid dysfunction and prior radioactive iodine treatment [4–7]. TAO is more common in women but is more severe in men [8]. Recent evidence indicates a possible role of orbital fibroblasts in the pathogenesis of TAO even though the reason for anatomic site-specific localization remains uncertain. Once activated by thyroid-stimulating immunoglobulins, fibroblasts proliferate and produce pro-inflammatory cytokines and extracellular matrix constituents [9]; this results in hygroscopic swelling of extracellular muscles and expansion of the adipose tissue. Three pathophysiological steps have been identified in the so-called “Cone model”: (a) expansion of rectus muscles and fat, forward displacement of extra-conal fat; (b) axial advancement of the globe and rectus muscle stretching; (c) impaired posterior venous drainage and reversal of conjunctival venous flow with eyelid edema [10].

Hyperthyroidism may also induce an increase in heart rate, cardiac output, and systolic blood pressure [11, 12]. In this setting, hemodynamic changes have been observed in many organs, including the eyes [13, 14]. A reduction in pulsatile ocular blood flow, pulse amplitude and pulse volume [15] and an increase in retinal blood flow have been observed in patients with Graves’ disease [16].

As the choroid is the main vascular layer of the eye, several studies investigated choroidal thickness (CT) changes in patients with TAO [17–21]. However, discrepancy in clinical findings and the clinical activity of the disease has been observed in various studies [17–21].

Recently, a new optical coherence tomography (OCT) parameter termed choroidal vascularity index (CVI) has been introduced to investigate the choroidal vasculature [22, 23]. It was obtained by binarization of optical coherence tomography images and was defined as the proportion of luminal area (LA) to total cross-sectional choroidal area (TCA) [24]. CVI gained increasing interest since it was observed to not be influenced by age, gender, refractive error, axial length or intraocular pressure (IOP) [24].

Therefore, our study aimed to assess changes in the choroidal vasculature using the CVI in eyes with TAO, and to compare the results with age- and sex-matched healthy controls. An additional objective was to evaluate the relation between CVI and clinical activity score, exophthalmometric value, diplopia status, gender and age.

Methods
In this cross-sectional single center study, we included 40 patients affected by TAO referred to Ophthalmology Unit by the Endocrinology Department of Pisa University Hospital. Forty healthy individuals, matched for age and sex, served as controls. This study received approval by the local Institutional Review Board (Comitato Etico, Area Vasta Nordovest, register number 18781) and was conducted in adherence to the tenets of the current version of the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013). All patients signed an informed consent form.

All subjects underwent a complete ophthalmological examination. The following data were collected: age, gender, visual acuity, intraocular pressure (IOP) (Goldmann applanation tonometry), biomicroscopy findings, clinical activity score (CAS), concomitant and previous therapy, exophthalmometric values and motility status. Ocular proptosis was measured by a Hertel exophthalmometer. The items included in the CAS grading scale were: (a) spontaneous retrobulbar pain; (b) pain on attempted upward or downward gaze; (c) eyelid erythema; (d) eyelid edema; (e) conjunctival hyperemia; (f) conjunctival chemosis; (g) inflammation of caruncle or plica. Assigning 1 point for each item, TAO was classified as active if CAS was ≥ 3 [25]. The presence of subjective diplopia in primary gaze position was graduated using Gorman score (0: no diplopia, 1: intermittent diplopia, 2: inconstant diplopia, and 3: constant diplopia) [26]. Inclusion criteria were diagnosis of Graves’ disease in the last 12 months; first episode of TAO; age between 25 and 45 years; euthyroidism in treatment with antithyroid drugs; refractive value (spherical equivalent) within the range – 3 diopters (D) to + 3 D. We excluded patients with a history of radiotherapy or thyroidectomy; previous treatment with corticosteroids in the 3 months before enrolment; any ocular or systemic disease that could interfere with the measurements of this study such as glaucoma, diabetic retinopathy, hypertensive retinopathy, previous vitreoretinal surgery, and retinal vein occlusion. The control group comprised of 40 healthy subjects with the same age range (25–45 years) and the same male: female proportion (4: 1) vs. the study group. They had no history of ocular or systemic disease and minimal refractive error (spherical equivalent) of ± 3 D.

Spectral-domain optical coherence tomography (SD-OCT, Spectralis; Heidelberg Engineering, Germany, Software version 6.9) was performed in all subjects using the enhanced-deep image (EDI) mode to obtain a better visualization of the choroid. A volume scan of 20° × 20° centered on the fovea was obtained for each eye. A single B-scan was an average of 20 frames and 240 μm apart from the next B-scan.

Subfoveal choroidal thickness (SFCT) was manually measured using the caliper tool embedded in the software of the instrument. Two trained masked examiners independently analyzed all the OCT scans and manually measured the SFCT, identified the choroidal boundaries and processed the images for binarization. The average of the measurements of the two examiners was considered for statistical analysis. Acquisitions were performed at the same time of the day (12: 00–14: 00) to avoid
diurnal variations. Images quality was checked just after acquisition and immediately repeated if necessary. Only scans with at least signal strength ≥ 6 and clearly identifiable choroid-scleral junction were taken for further analysis.

Binarization of images

The same foveal scan used for CT measurement was processed using the open-source software ImageJ (version 1.52; National Institutes of Health, USA, http://imagej.nih.gov/ij). The polygon tool was used to select the TCA. The selection was added to the region of interest (ROI) manager. The image was then downgraded to 8-bit and adjusted with Niblack auto local threshold. Color threshold was used to select the LA which was added to the ROI manager. CVI was calculated as the proportion of LA to TCA. Stromal area (SA) was calculated by subtracting LA from TCA (Fig. 1).

Statistical analysis

Statistical analysis was performed using the SPSS software version 20.0 for Windows (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as mean ± standard deviation, and quantitative variables were expressed as frequency (%). The normality of distribution of data was assessed using Kolmogorov-Smirnov and Shapiro-Wilk tests. Intraclass Correlation Coefficient (ICC) was calculated to evaluate the correlation between right and left eyes. Differences in SFCT, TCA, SA, LA and CVI were assessed applying a two-side independent sample t-test. Univariate linear regression analysis was performed indicating CVI and SFCT as dependent variables and TCA, CAS, exophthalmometric value, Inami value, diplopia grade, gender and age as independent variables. Covariates with a P-value < 0.2 in univariate analyses were included in the multivariable analysis. P values < 0.05 were considered statistically significant; 95% confidence intervals (CI) were presented.

Results

Both eyes of 80 patients, 21 males and 59 females, were included in this study. Forty cases were diagnosed with TAO and 40 subjects served as controls. Mean age was 39.30 ± 4.54 years (range 30–45 years) and 37.45 ± 4.44 years (range 28–45 years) respectively; the difference was not significant (P = 0.069, t-test). Since there was a strong correlation between right and left eye variables, only data of the right eyes were included in the statistical analysis. Indeed, the ICC value for SFCT was 0.947 (95% CI 0.899–0.972, P < 0.001) and for CVI was 0.953 (95% CI 0.906–0.974, P < 0.001).

![Fig. 1 Binarization and identification of the luminal and stromal areas of the choroid. Spectral-domain optical coherence tomography (SD-OCT) acquired using enhanced-depth image (EDI) mode. a Original subfoveal scan; b The image was downgraded to 8-bit and Niblack auto local threshold was applied; c Color threshold was used to select luminal area; d Overlay of the region of interest on the original image](image)
Mean values of TCA, LA, SA, CVI and SFCT are displayed in Table 1.

No significant differences were observed in SFCT, TCA and SA comparing patients with TAO and healthy controls (all $P > 0.05$). LA was significantly higher in TAO subjects when compared with controls ($P = 0.045$).

Mean CVI significantly differed between TAO patients ($64.78 \pm 3.28\%$, range: 52.60–72.13%) and healthy controls ($62.19 \pm 4.44\%$, range: 51.89–70.23%) ($P = 0.004$, t-test). We performed a subgroup analysis considering only patients with CAS $\geq 3$ (28 subjects, 23 females). No significant differences were observed between patients with active TAO and controls in TCA ($0.58 \pm 0.18\ mm^2$ and $0.53 \pm 0.10\ mm^2$, respectively), SA ($0.207 \pm 0.055\ mm^2$ and $0.208 \pm 0.054\ mm^2$, respectively) and SFCT ($316.68 \pm 74.94\ mm^2$ and $281.93 \pm 49.75\ mm^2$, respectively) ($all P > 0.05$). LA ($0.38 \pm 0.13\ mm^2$ and $0.32 \pm 0.06\ mm^2$, respectively, $P = 0.046$) and CVI ($64.37 \pm 3.34\%$ and $61.42 \pm 4.90\%$, respectively, $P = 0.011$) were significantly higher in patients with active TAO. Mean exophthalometric value in TAO patients was $22.13 \pm 2.86$ (95% CI 21.29–23.13); mean CAS was $3.48 \pm 1.78$, (95% CI 2.90–4.05); mean Inami value was $107.03 \pm 4.25$, (95% CI 105.66–108.39). Active TAO was present in 28/72.13% and healthy controls (62.19 ± 4.44, range: 51.89–70.23%) ($P = 0.004$). LA was significantly higher in patients with active TAO than in healthy controls, despite similar SFCT. Increased CT was reported in eyes with TAO by different studies in the last 4 years, summarized in Table 3 [17–21].

Starting from the segmentation method proposed by Sonoda and colleagues [35, 36], Agrawal and co-workers proposed the introduction of a novel OCT marker called CVI, defined as the proportion of LA to TCA [24]. CVI was analyzed in several studies in normal and pathologic conditions including age-related macular degeneration, central serous chorioretinopathy, open-angle glaucoma, Type 2 diabetes and Vogt-Koyanagi-Harada disease [37–41].

In this study, we found that CVI was higher in eyes with TAO than in healthy controls, despite similar SFCT. Increased CT was reported in eyes with TAO by different studies in the last 4 years, summarized in Table 3 [17–21].

CT was measured in the subfoveal region and at different distances from the fovea. In our study, no significant difference in SFCT was found comparing TAO patients and age- and sex-matched healthy controls, though a statistical trend ($P = 0.055$) was found in the subgroup analysis considering only patients with CAS $\geq 3$.

### Discussion

Located between the retina and sclera, the choroid is the main vascular layer of the eye. It provides oxygen and nourishment to the fovea and the outer layers of the retina. It is composed of three different zones: the choriocapillaris, the Sattler’s layer with medium size vessels and the Haller’s layer, adjacent to the scleral boundary, with large vessels [27]. The choroid is supplied by posterior ciliary arteries originating from the ophthalmic artery that derives from the internal carotid artery [28]. Reflux blood is collected by the vortex veins, tributary of the ophthalmic vein. Due to the valve-less structure of the ophthalmic veins, choroidal vasculature may be influenced by systemic conditions that have an impact on venous blood flow [29].

Several methods have been proposed to investigate choroidal changes in many pathological conditions, such as histopathological assessment, pulsatile blood flow tonometry, doppler flowmetry, wavelet augmented ultrasound, fluorescein and indocyanine green angiography [30–32]. However, their applicability in daily practice and research settings was limited by the lack of reliability and repeatability or inadequate quantitative parameters for analysis [30].

Thanks to technological advances, OCT has gained a leading role as it allows us to obtain high-resolution in vivo images in a fast and non-invasive way. In EDI mode acquisition, the lens of the instrument is moved closer to the eye and the zero-delay line is settled beside the choroid; this improves the visualization of the choroid and inner sclera [33]. SFCT measurements obtained with EDI OCT have shown a high intra- and inter-observer reproducibility [34].

| Parameter                  | TAO group      | Control group | $P$  |
|---------------------------|----------------|---------------|------|
| Subfoveal choroidal thickness ($\mu m$) | $308.08 \pm 73.37$ | $288.90 \pm 58.32$ | 0.200 |
| Total choroidal area ($mm^2$) | $0.61 \pm 0.21$ | $0.55 \pm 0.11$ | 0.153 |
| Luminal area ($mm^2$) | $0.39 \pm 0.14$ | $0.34 \pm 0.72$ | 0.045 |
| Stromal area ($mm^2$) | $0.21 \pm 0.06$ | $0.21 \pm 0.05$ | 0.927 |
| Choroidal vascularity index (%) | $64.78 \pm 3.28$ | $62.19 \pm 4.44$ | 0.004 |

$TAO = thyroid-associated ophthalmopathy$
However, the smaller sample size may limit the statistical power. A possible explanation may be found in the heterogeneity of the study population since most of the studies reported a wider age range (20–70 years) compared with our subjects (25–45 years). A negative correlation has been reported between CT and age, with approximately a mean CT decrease of 1.5 μm for each year’s increase in age [42]. Eyes of younger individuals may better compensate for modification in choroidal blood flow due to systemic factors and inflammation. Furthermore, in long-lasting disease, the chronic inflammatory insult may result in choroidal vasculopathy and atrophic involution [43]. Recently, Del Noce et al. observed differences in choroidal vascular blood flow in patients with TAO using Angio-OCT when compared with healthy controls [44]. The use of CT as a biomarker has some intrinsic limitations including its circadian fluctuations and its dependence on gender, age and refractive status. Contrarily, current research indicates that CVI is less influenced by physiologic parameters and has minor variability than CT [23].

The choroid is composed of blood vessels surrounded by extracellular matrix. Modifications in CT did not indicate which component was more affected and in what proportion. Furthermore, an increase in one component may be compensated by a reduction of the other. For these reasons, CT is only unfairly representative of the complete choroidal structural modifications.

CVI is the proportion of choroid vasculature to overall choroidal area. An increase in CVI may depend on either an increase in the diameter of the choroidal blood vessels or in the number of blood vessels within a selected region. An increase in retinal blood flow has been observed in active TAO patients [13, 15]. Furthermore, Graves’ orbitopathy is characterized by swelling of extraocular muscles and orbital tissue and fat. The expansion of intraorbital contents in TAO patients is hindered by the inextensible rigid bony walls of the orbit. Consequently, the eyeball is compressed by the expansion of fat and muscles; this may have implications for IOP levels and venous drainage. Increased values of IOP have been documented in patients with TAO when compared with healthy controls [45]. Reverse blood flow of the superior ophthalmic vein and venous stasis in the orbit have been observed in patients with TAO [46–48]. CVI was found to be higher in other clinical

| Parameter                  | Univariate analysis | Multivariate analysis |
|----------------------------|---------------------|-----------------------|
|                           | β       | 95% CI | P       | β       | 95% CI | P       |
| SFCT                      |         |        |         |         |        |         |
| Age                       | −0.017  | −5.58 – 5.03 | 0.917 | −         |         |         |
| Gender                    | −0.018  | −60.17 – 53.77 | 0.910 | −         |         |         |
| Diplopia (yes vs. no)     | 0.071   | −38.45 – 59.60 | 0.665 | −         |         |         |
| CAS                       | 0.281   | −1.389 – 24.55 | 0.079 | 0.266   | −1.99 – 23.89 | 0.095 |
| Exophthalmometry          | −0.011  | −8.69 – 8.11 | 0.944 | −         |         |         |
| Inami                     | −0.167  | −8.46 – 2.69 | 0.302 | −         |         |         |
| TCA                       | −0.183  | 0.00 – 0.00 | 0.258 | −         |         |         |
| LA                        | −0.211  | 0.00 – 0.00 | 0.191 | −0.190   | 0.00 – 0.00 | 0.229 |
| SA                        | −0.108  | 0.00 – 0.00 | 0.506 | −         |         |         |
| CVI                       |         |        |         |         |        |         |
| Age                       | 0.060   | −0.19 – 0.28 | 0.713 | −         |         |         |
| Gender                    | 0.023   | −2.37 – 2.72 | 0.889 | −         |         |         |
| Diplopia (yes vs. no)     | 0.016   | −2.09 – 2.30 | 0.924 | −         |         |         |
| CAS                       | −0.231  | −1.01 – 0.16 | 0.152 | 0.016   | −0.13 – 0.19 | 0.699 |
| Exophthalmometry          | −0.095  | −0.48 – 0.26 | 0.599 | −         |         |         |
| Inami                     | 0.206   | −0.09 – 0.41 | 0.202 | −         |         |         |
| TCA                       | 0.402   | 0.00 – 0.00 | 0.010 | −6.307   | 0.00 – 0.00 | <0.001 |
| LA                        | 0.513   | 0.00 – 0.00 | 0.001 | 6.757    | 0.00 – 0.00 | <0.001 |
| SA                        | 0.123   | 0.00 – 0.00 | 0.450 | −         |         |         |
| SFCT                      | −0.322  | −0.02 – 0.00 | 0.043 | −0.057   | −0.006 – 0.001 | 0.189 |

SFCT = subfoveal choroidal thickness; CAS = clinical activity score; TCA = total choroidal area; LA = lumen area; SA = stromal area; CVI = choroidal vascularity index; CI = confidence interval, significant P values are in bold.
conditions characterized by increased venous pressure such as in patients with carotid-cavernous fistula [49].

In this study, we did not find any correlation between CVI or SFCT and disease-related ocular parameters such as CAS and exophthalmometric values. This is in contrast with the results of Bruscolini and colleagues [18], Çalışkan and colleagues [50] and Özkan and colleagues [19], but is consistent with the findings of Yu and Zhang [21], and partially concords with Lai and co-workers [20] and Çalışkan and co-workers [17]. CT appeared to not be correlated with systemic parameters including disease duration, mean blood pressure, age, gender and thyroid hormone levels.

Exophthalmometric values of healthy subjects vary among populations depending on ethnicity, ranging from 16.5 mm in White men to 18.5 mm in Black men. Values in women are lower: 15.4 mm and 17.8 mm, respectively [51]. Asians have lower exophthalmometric values than Caucasians [52, 53], partially imputable to a tighter orbital septum limiting forward movement of the globe [54]. Exophthalmometric values of TAO patients were higher than in the general population as a result of the propulsive forces that occur in the orbit of TAO patients.

A limitation of this study is that the identification of the choroidal boundaries and the measurement of CT are subjective and operator-dependent processes. Furthermore, measurement of CVI was performed in a 2D scan image across the fovea. A volume scan covering the entire macula might provide more information and reduce sampling error.

**Table 3** Synthesis of previously reported findings regarding choroidal thickness in patients with Graves’ disease

| Authors                  | Methods                                      | Nationality of the study population | Results                                                                 | Correlation with ocular parameters                                                                 | Correlation with systemic parameters                                                                 | Study limitations                                                                 |
|--------------------------|----------------------------------------------|-------------------------------------|------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| Çalışkan and Akay 2018 [9] | Subfoveal CT; at 500 μm, 1000 μm, and 1500 μm temporal and nasal to the fovea | Turkish                             | Increased subfoveal, mean and temporal CT; no difference in nasal CT | Correlation with VISA score No correlation with exophthalmometry, axial length                         | No correlation with disease duration, mean blood pressure                                        | Superior or inferior CT not measured                                                 |
| Bruscolini et al. 2018 [10] | Subfoveal CT                                  | Italian                             | Increased subfoveal CT                                                | Correlation with CAS and exophthalmometry                                                               | No correlation with disease duration                                                                  | Small sample size (n = 18); lack of perifoveal measurements                           |
| Özkan et al. 2016 [11]    | Subfoveal CT                                  | Turkish                             | Increased subfoveal CT                                                | Correlation with CAS and elongated VEP P100                                                           | N/A                                                                                                  | Only SFCT was examined; small sample size                                           |
| Lai et al. 2019 [12]      | Subfoveal CT; at 1 mm and 2 mm nasal, temporal, inferior and superior to the fovea; peripapillary region | Chinese                             | Increased CT in all point except at 2 mm inferior to the fovea and at peripapillary region | Association with axial length, exophthalmometry and BCVA No association with IOP and CAS              | No association with age, gender, duration of TAO, history of smoking                           | Most patients with CAS < 4                                                          |
| Yu and Zhang 2018 [13]     | Subfoveal CT; at 1500 μm and 3000 μm nasal and temporal to the fovea | Chinese                             | CT increased in all points                                           | No relationship with CAS, degree of exophthalmos                                                       | No relationship with T3, T4, TSH, TRAb levels                                                     | Lack of superior and inferior measurements                                         |
| Çalışkan et al. 2017 [14]  | Subfoveal CT; at 1.5 mm and 3.0 mm nasal and temporal to the fovea | Turkish                             | Increased CT                                                         | Correlation with CAS, IOP                                                                                 | Correlation with age, disease activity                                                              | Wide range of patient age (21–65 years)                                               |

VISA = vision, inflammation, strabismus, and appearance; CT = choroidal thickness; CAS = clinical activity score; VEP = visual-evoked potential; N/A = not applicable; SFCT = subfoveal choroidal thickness; BCVA = best-corrected visual acuity; IOP = intraocular pressure; T3 = triiodothyronine; T4 = tetraiodothyronine; TRAb = thyroid-stimulating hormone receptor antibody; TSH = thyroid-stimulating hormone

**Conclusions**

CVI was found to be increased in eyes of subjects with TAO. CVI was significantly associated with TCA, LA and SFCT in univariate analysis and with TCA and LA in multivariable linear regression analysis. No association was found between either CVI or SFCT and age, gender, presence of diplopia, CAS, exophthalmometry and Inami. This is the first study that has compared CVI in eyes with TAO and healthy controls and has assessed its association with clinical features; it can, therefore, serve as a starting point for further prospective research.

**Abbreviations**

CAS: Clinical activity score; CI: Confidence interval; CT: Choroidal thickness; CVI: Choroidal vascularity index; EDI: Enhanced deep image; ICC: Intraclass correlation coefficient; IOP: Intraocular pressure; LA: Luminal area; ROC: Region of interest; SA: Stroma area; SD-OCT: Spectral domain – optical coherence tomography; SFCT: Subfoveal choroidal thickness; TAO: Thyroid-associate ophtalmopathy; TCA: Total choroidal area

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Authors’ contributions
PL was a major contributor in writing the manuscript. BM, II and GC contributed to the acquisition of data. MP and PL analyzed the data. PL, MM and GC contributed to the conception of the study. MF and MN revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study received approval by the local Institutional Review Board (Comitato Etico, Area Vasta Nordovest, register number 18781) and was conducted in adherence to the tenets of the current version of the Declaration of Helsinki (84th WMA General Assembly, Fortaleza, Brazil, October 2013).

Consent for publication
All patients signed an informed consent form.

Competing interests
The authors declare that they have no competing interests.

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References
1. Smith TJ, Hedges Jr L. Graves’ disease. N Engl J Med. 2016;375(16):1552–65.
2. Bahn RS. Graves’ ophthalmopathy. N Engl J Med. 2010;362(8):726–38.
3. Łacheta D, Miśkiewicz P, Glusko A, Nowicka G, Struga M, Kantor I, et al. Immunological aspects of Graves’ ophthalmopathy. Biomed Res Int. 2019;2019:7453260.
4. Stan MN, Bahn RS. Risk factors for development or deterioration of Graves’ ophthalmopathy. Thyroid. 2010;20(7):777–83.
5. Prummel MF, Wiersinga WM, Mourtis MP, Koornneef L, Berghoit A, van der Gaag R. Effect of abnormal thyroid function on the severity of Graves’ ophthalmopathy. Arch Intern Med. 1990;150(5):1098–101.
6. Thornton J, Kelly SP, Harrison RA, Edwards R. Cigarette smoking and thyroid eye disease: a systematic review. Eye (Lond). 2007;21(9):1135–45.
7. Stan MN, Durkić JM, Brits JP, Bhagra S, Thapa P, Bahn RS. Cohort study on radiative iodine-induced hypothyroidism: implications for Graves’ ophthalmopathy and optimal timing for thyroid hormone assessment. Thyroid. 2013;23(5):620–2.
8. Perros P, Cribbé AL, Matthews JN, Kendall-Taylor P. Age and gender influence the severity of thyroid-associated ophthalmopathy: a study of 101 patients attending a combined thyroid-eye clinic. Clin Endocrinol (Oxf). 1993;38(4):367–72.
9. Pritchard J, Han R, Horst N, Cruikshank WW, Smith TJ. Immunoglobulin activation of T cell chemotacticant expression in fibroblasts from patients with Graves’ disease is mediated through the insulin-like growth factor I receptor pathway. J Immunol. 2003;170(2):6348–54.
10. Meyer P, Das T, Ghadiri N, Murthy R, Theodoropoulou S. Clinical immunopathophysiology of thyroid eye disease: the cone model. Eye (Lond). 2019;33(2):244–53.
11. Jabbar A, Pingitore A, Pearce SH, Zaman A, Iervasi G, Razvi S. Thyroid hormones and cardiovascular disease. Nat Rev Cardiol. 2017;14(1):39–55.
12. Klein L, Danzi S. Thyroid disease and the heart. Curr Probl Cardiol. 2016;41(2):65–92.
13. Yanik B, ComkBıyar I, Acaroglu G, Hekimoğlu B. Graves’ ophthalmopathy: comparison of the Doppler sonography parameters with the clinical activity score. J Clin Ultrasond. 2005;33(3):87–80.
14. Alp MN, Ozgen A, Can I, Cakar P, Gunalp I. Colour Doppler imaging of the orbital vasculature in Graves’ disease with computed tomographic correlation. Br J Ophthalmol. 2000;84(9):1027–30.
15. Tsai CC, Kau HC, Koo SC, Lin MW, Hsu WM, Liu JH, et al. Pulsatile ocular blood flow in patients with Graves’ ophthalmopathy. Eye (Lond). 2005;19(2):159–62.
16. Perri P, Campa C, Costagliola C, Incoavia C, D’Angelo S, Sebastiani A. Increased retinal blood flow in patients with active Graves’ ophthalmopathy. Curr Eye Res. 2007;32(11):895–80.
17. Cagiltay E, Akay F. The increment of choroidal thickness in euthyroid Graves’ ophthalmopathy: is it an early sign of venous congestion? J Ophthalmol. 2018;2018:5891331.
18. Bruscolini A, La Cava M, Gharbiya M, Sacchetti M, Restivo L, Nardella C, et al. Management of patients with Graves’ disease and orbital involvement: role of spectral domain optical coherence tomography. J Immunol Res. 2018;2018:1454616.
19. Özkan B, Koçer ÇA, Altıntaş O, Karabas L, Acar AZ, Yüksel N. Choroidal changes observed with enhanced depth imaging optical coherence tomography in patients with mild Graves orbitopathy. Eye (Lond). 2016;30(7):917–24.
20. Liu FH, Iao TWU, Ng DSC, Young AL, Leung J, Ju A, et al. Choroidal thickness in thyroid-associated orbitopathy. Clin Exp Ophthalmol. 2019;47(7):918–24.
21. Yu N, Zhang Y. Analysis in choroidal thickness in patients with Graves’ ophthalmopathy using spectral-domain optical coherence tomography. J Ophthalmol. 2018;2018:3529395.
22. Agrawal R, Ding J, Sen P, Rousselot A, Chan A, Nivison-Smith L, et al. Exploring choroidal angioarchitecture in health and disease using choroidal vascularity index. Prog Retin Eye Res. 2020;77:100829.
23. Iovino C, Pellegrini M, Benabei F, Borelli E, Sacconi S, Goveo A, et al. Choroidal vascularity index: an in-depth analysis of this novel optical coherence tomography parameter. J Clin Med. 2020;9(2):595.
24. Agrawal R, Gupta P, Tan KA, Cheung CM, Wong TY, Cheng CY. Choroidal vascularity index as a measure of vascular status of the choroid measurements in healthy eyes from a population-based study. Sci Rep. 2016;6:21090.
25. Mourtis MP, Prummel MF, Wiersinga WM, Koornneef L. Clinical activity score as a guide in the management of patients with Graves’ ophthalmopathy. Clin Endocrinol (Oxf). 1997;47(1):9–14.
26. Bartalena L, Tanda ML. Clinical practice. Graves’ ophthalmopathy. N Engl J Med. 2009;360(10):994–1001.
27. Almi A, Bill A. Ocular and optic nerve blood flow at normal and increased intraocular pressures in monkeys. (Macaque inu): a study with radioactively labelled microspheres including flow determinations in brain and some other tissues. Exp Eye Res. 1973;15(1):115–29.
28. Toma N. Anatomy of the ophthalmic artery: embryological consideration. Neurol Med Chir (Tokyo). 2016;56(10):585–91.
29. Monteiro MLR, Angotti-Neto H, Benabou JE, Betnjane AJ. Color Doppler imaging of the superior ophthalmic vein in different clinical forms of Graves’ orbitopathy. Jpn J Ophthalmol. 2008;52(6):485–8.
30. Spaide RF, Yannuzzi LA, Slakter JS, Sorenson J, Orlach DA. Indocyanine green videaoangiography of idiopathic polypoidal choroidal vasculopathy. Retina. 1995;15(2):100–10.
31. Chen SJ, Cheng CY, Lee AF, Lee FL, Chou JC, Hsu WM, et al. Pulsatile ocular blood flow in asymmetric exudative age related macular degeneration. Br J Ophthalmol. 2001;85(12):1411–5.
32. Coleman DJ, Silverman RH, Rondeau MJ, Lloyd HO, Khanifar AA, Chan RV. Age-related macular degeneration: choroidal ischaemia? Br J Ophthalmol. 2013;97(8):1020–3.
33. Margolis R, Spaide RF. A pilot study of enhanced depth imaging optical coherence tomography of the choroid in normal eyes. Am J Ophthalmol. 2009;147(5):811–5.
34. Shao L, Xu L, Chen CX, Yang LH, Du KF, Wang S, et al. Reproducibility of subfoveal choroidal thickness measurements with enhanced depth imaging by spectral-domain optical coherence tomography. Invest Ophthalmol Vis Sci. 2013;54(1):230–3.
35. Sonoda S, Sakamoto T, Yamashita T, Shirasawa M, Uchino E, Terasaki H, et al. Choroidal structure in normal eyes and after photodynamic therapy
determined by binarization of optical coherence tomographic images. Invest Ophthalmol Vis Sci. 2014;55(6):3893–9.

36. Sonoda S, Sakamoto T, Yamashita T, Uchino E, Kawano H, Yoshihara N, et al. Luminal and stromal areas of choroid determined by binarization method of optical coherence tomographic images. Am J Ophthalmol. 2015;159(6):1123–31.e1.

37. Agrawal R, Chhablani J, Tan KA, Shah S, Sarvaiya C, Banker A. Choroidal vascularity index in central serous chorioretinopathy. Retina. 2016;36(9):1646–51.

38. Agrawal R, Li LK, Naikhatve V, Khandelval N, Mahendra N. Coroidal vascularity index in Vogt-Koyanagi-Harada disease: an EDI-OCT-derived tool for monitoring disease progression. Transl Vis Sci Technol. 2016;5(4):7.

39. Kim M, Ha MJ, Choi SY, Park YH. Choroidal vascularity index in type-2 diabetes analyzed by swept-source optical coherence tomography. Sci Rep. 2018;8(11):70.

40. Park Y, Cho KJ. Choroidal vascular index in patients with open angle glaucoma and preperimetric glaucoma. PLoS One. 2019;14(3):e0213336.

41. Wei X, Ting DSW, Ng WY, Khandelval N, Agrawal R, Cheung CMG. Choroidal vascularity index: a novel optical coherence tomography-based parameter in patients with exudative age-related macular degeneration. Retina. 2017;37(6):1120–5.

42. Pongpachareonnont P, Somkijrungroj T, Assavapongpaiboon B, Chitamara T, Chumtarapap M, Sunuwanakorn D. Foveal and parafoveal choroidal thickness pattern measuring by swept source optical coherence tomography. Eye (Lond). 2019;33(9):1443–51.

43. Steiner M, Estevez-Ortega MD, Muñoz-Fernández S. Choroidal and retinal thickness in systemic autoimmune and inflammatory diseases: a review. Surv Ophthalmol. 2019;64(6):757–69.

44. Del Noce C, Vagge A, Nicolò M, Traverso CE. Evaluation of choroidal thickness and choroidal vascular blood flow in patients with thyroid-associated orbitopathy (TAO) using SD-OCT and Angio-OCT. Graefes Arch Clin Exp Ophthalmol. 2020;258(5):1103–7.

45. Eslami F, Borzouei S, Khanlarzadeh E, Seif S. Prevalence of increased intraocular pressure in patients with Graves’ ophthalmopathy and association with ophthalmic signs and symptoms in the north-west of Iran. Clin Ophthalmol. 2019;13:1353–9.

46. Nakase Y, Osanai T, Yoshikawa K, Inoue Y. Color Doppler imaging of orbital venous flow in thyrotoxic optic neuropathy. Jpn J Ophthalmol. 1994;38(1):180–6.

47. Konuk O, Onaran Z, Ozhan Oktar S, Yucel C, Unal M. Intraocular pressure and superior ophthalmic vein blood flow velocity in Graves’ orbitopathy: relation with the clinical features. Graefes Arch Clin Exp Ophthalmol. 2009;247(11):1535–9.

48. Somer D, Ozkan SB, Ozdemir H, Attiba S, Soyver MF, Duman S. Colour Doppler imaging of superior ophthalmic vein in thyroid-associated eye disease. Jpn J Ophthalmol. 2002;46(3):341–5.

49. Inam O, Arat YO, Yavas GF, Arat A. Retinal and choroidal optical coherence tomography findings of carotid cavernous fistula. Am J Ophthalmol. 2019;206:264–73.

50. Çalıkkan S, Acar M, Güral C. Choroidal thickness in patients with Graves’ ophthalmopathy. Curr Eye Res. 2017;42(3):484–90.

51. Migliori ME, Gladstone GJ. Determination of the normal range of exophthalmometric values for black and white adults. Am J Ophthalmol. 1984;98(4):438–42.

52. Lim NC, Sundar G, Amrith S, Lee KO. Thyroid eye disease: a southeast Asian experience. Br J Ophthalmol. 2015;99(4):512–8.

53. Lim SL, Lim AK, Mumtaz M, Hussein E, Wan Bebakar WM, Khir AS. Prevalence, risk factors, and clinical features of thyroid-associated ophthalmopathy in multiethnic Malaysian patients with Graves’ disease. Thyroid. 2008;18(12):1297–301.

54. Ersan I, Oltulu R, Altunkaya O, Saritav G, Arkan S, Donbaloglu M, et al. Relationship of inferior oblique overaction to macular and subfoveal choroidal thickness. J AAPOS. 2015;19(1):21–3.