Retina and Nerve Fiber Layer Thickness in Eyes with Thyroid-Associated Ophthalmopathy

Sharon Blum Meirovitch MD, Igal Leibovitch MD, Anat Kesler MD, David Varssano MD, Amir Rosenblatt MD MPH and Meira Neuderfer MD

Department of Ophthalmology, Tel Aviv Sourasky Medical Center, affiliated with Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

ABSTRACT: **Background:** Thyroid-associated ophthalmopathy (TAO) is an inflammatory disease that affects the thyroid gland and the eye orbit. Of patients with TAO, 3%–5% have severe sight-threatening disease due to optic neuropathy. Optical coherence tomography (OCT), the non-invasive imaging technology that yields high-resolution cross-sectional images of the retina, provides qualitative and quantitative data on the retina.

Objectives: To apply this technique to quantitatively assess retinal nerve fiber layer (RNFL) and macular ring thicknesses in healthy subjects and in patients with TAO to determine their relationship to the severity of the orbital disease.

Methods: The study group comprised all patients in the ophthalmology clinic who were diagnosed with TAO and underwent OCT imaging as part of their ocular examination; and healthy patients who volunteered to undergo OCT examination served as controls. Results of the complete ophthalmologic examination and OCT findings were collected from medical files, including the thickness of the RNFL and the macula.

Results: The study comprised 21 patients and 41 healthy controls. TAO patients exhibited RNFL thickening and inner macula thinning compared to healthy subjects. Mean RNFL thickness was correlated with the severity of the orbital disease.

Conclusions: The OCT findings suggest that the retina is involved in TAO, probably as early as the subclinical stage. This highlights the ability of OCT to identify retinal changes earlier and far more accurately than is detected today, enabling earlier diagnosis and more timely treatment to prevent severe visual sequelae.

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KEY WORDS: thyroid-associated ophthalmology (TAO), optical coherence tomography (OCT), macular thickness, retinal nerve fiber layer (RNFL) thickness, VISA classification

Thyroid-associated ophthalmopathy (TAO) is an inflammatory disease that typically affects the thyroid gland and the orbit. TAO usually presents bilaterally and often asymmetrically [1]. The ocular manifestations in TAO are mild in the majority of cases, although approximately 5% of patients with Graves' disease and up to 9% of patients in a tertiary care setting have optic neuropathy [2,3]. This emergent condition requires prompt evaluation and treatment to prevent possible permanent loss of vision. The current leading theory for the cause of optic nerve damage points to the orbital mechanical compression by the extraocular muscles and soft tissue expansion that are seen in TAO. Recent studies supported this etiology by demonstrating the relationship between muscle enlargement and the appearance of optic neuropathy [4,5].

It is sometimes difficult to diagnose optic nerve involvement in TAO because of the variations in clinical appearances. Visual acuity, visual field, color vision and optic disk configuration are all subject to change, and the findings are not necessarily congruent [2]. Reseasrch has dealt with the thickness of the macular ring and the retinal nerve fiber layer (RNFL) in TAO patients, with conflicting results. Wei et al. [6] reported that enlargement of the extraocular muscles can be an early sign of optic neuropathy, but they found no correlation between the cross-sectional areas of the rectus muscles and the thickness of the RNFL, or between the thickness of the RNFL and other visual function parameters (such as retinal sensitivity, P100 value of visual evoked potential, and color sensation test). Forte and colleagues [7] also found no significant reduction in RNFL thickness in patients with Graves' ophthalmopathy and ocular hypertension > 23 mmHg, and reported a low correlation between RNFL thickness and visual field abnormalities.

Optical coherence tomography (OCT) is a relatively new non-invasive, trans-pupillary imaging technology that provides high-resolution cross-sectional images of the retina, including the RNFL. The technology gives qualitative and quantitative data on the macular ring and the RNFL and has already proven useful for assessing retinal diseases and optic disk pathologies. OCT has been used extensively to diagnose and monitor RNFL loss in patients with glaucoma, and to evaluate macular holes, macular edema, age-related macular degeneration, and other disorders [8,9].

The present study was undertaken to quantitatively assess RNFL and macular ring thicknesses by OCT in healthy subjects and patients with TAO, and to determine whether there is a correlation between those parameters and the severity of the orbital disease.

PATIENTS AND METHODS

In this analytic case-control study, the study group consisted of patients with TAO at different stages of the disease; the control group comprised healthy subjects with no ocular pathologies who had been evaluated at the ophthalmology division of our medical center where they underwent clinical examination and OCT imaging. The data pertinent to this study were retrieved from a computerized database at the Tel Aviv Sourasky Medical Center.

Only adult candidates with good quality OCT scans were included. Subjects were excluded if they presented any other ocular or systemic disease that could affect the optic nerve or thickness of the retina, or if they had a contraindication to pupil dilatation or hypersensitivity or intolerance to topical anesthetics or mydriatics.

Demographics and anamnestic data on risk factors, medical history, symptoms and treatment were collected. Comprehensive ophthalmic data were obtained from the patients' clinical examinations, including the extent of the proptosis, eyelid retraction, eyelid edema and hyperemia, extraocular muscle movements, optic nerve functioning (best-corrected visual acuity, color vision, relative afferent pupillary defect), slit-lamp biomicroscopy, intraocular pressure (IOP) measurement, and dilated funduscopic examination of the optic disk. The study protocol was approved by the institutional review board.

EVALUATION OF TAO SEVERITY

Severity of TAO was determined by the VISA classification [10], which grades clinical severity and activity according to both subjective and objective inputs. It separates the various clinical features of TAO into four discrete parameters: V (vision, identify the presence of dysthyroid optic neuropathy), I (orbital soft tissue inflammation or congestion), S (strabismus, motility restriction), and A (appearance, exposure).

OCT EVALUATION

The commercially available Stratus OCT-3 (software version 3.0, Carl Zeiss Meditec Inc., Dublin, CA, USA) was used for ocular imaging in subjects with mydramide-dilated pupils. The macular thickness and fast RNFL programs were used to assess the retinal and RNFL thicknesses. When more than one image was available for a patient, we chose the one that was taken close in time to the relevant ophthalmic examination. Both eyes of each subject were imaged for the study.

The macular thickness protocol algorithm differentiates between three macular zones: the fovea, the inner macular ring, and the outer macular ring. The inner and outer macular rings are further divided into four quadrants by two diagonal lines. Thus, a total of nine areas are available for analysis on a "topographic" map. We manually calculated the total thickness of the inner and outer macular rings and the mean macular thickness (the mean of the macular thickness measurements excluding the fovea) for each patient.

The fast RNFL protocol comprises three scans from each eye, with each scan consisting of 256 test points measured along a nominal 1.73 mm radius circle on the peripapillary RNFL using a single alignment and capture. The thickness of each test point was determined by averaging the three measurements. This protocol allows a quantitative measurement of the RNFL thickness around the optic disk. We evaluated average thickness (360° measure), temporal quadrant thickness (316°–45°), superior quadrant thickness (46°–135°), nasal quadrant thickness (136°–225°), and inferior quadrant thickness (226°–315°). Stratus OCT parameters and the data from the ophthalmic examination were considered as the dependent variables.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS version 21.0 (IBM, Armonk, NY). The inner and outer macular ring thicknesses and the RNFL thickness were compared between the two groups by an independent sample *t*-test. Correlation between the severity of the orbital disease and the macular ring and RNFL thicknesses was analyzed by the parametric Pearson correlation test and the Spearman non-parametric correlation test. All tests were two-tailed, and the threshold for statistical significance was defined as $P \leq 0.05$.

RESULTS

A total of 115 eyes were evaluated, 42 in the study group and 73 in the control. The study group comprised 4 males and 17 females with an average age of 44.1 years (range 19–64 years) and the control group 9 males and 32 females with an average age of 42.9 years (range 19–72 years). RNFL thickness was available for 41 eyes in the study group and 73 in the control group. Macular thickness was available for 40 eyes of the study group and 63 of the control group. The study group consisted of patients with TAO in different stages of the disease; the median VISA score was 5, with the severity score ranging from 1 to 17 [Table 1].

There was a significant thickening of the RNFL and the superior, inferior and nasal RNFL quadrants in eyes of TAO patients compared to control subjects. There was no significant difference in the temporal quadrant. There was a significant inner macula thinning in eyes of TAO patients compared to control subjects. A thinning of the outer macula ring in eyes of TAO patients compared to control subjects was not statistically significant, although the observed trend was in line with the inner macular thinning [Figures 1A and 1B].

A significant linear correlation was found between the VISA score and the overall mean RNFL thickness in both eyes ($R = 0.732$, $P < 0.001$). Furthermore, this correlation was still significant ($R = 0.578$, $P = 0.013$) even after excluding a suspected

Table 1. Demographic data and distribution of TAO stages, according to the VISA classification, in the study and control groups

Variable	VISA score	Study group (N=21)	Control group (N=41)	*P* value
Age (years), mean ± SD		44.19 ± 14.21	42.90 ± 14.65	0.7415
Gender, female N (%)		17 (80.95%)	32 (78.05%)	0.790
VISA score Median (range) Score, N (%)	 1 2 3 4 5 9 11 17	5 (1–17) 1 (4.8%) 3 (14.3%) 1 (4.8%) 2 (9.5%) 6 (28.6%) 1 (4.8%) 6 (28.6%) 1 (4.8%) NAION	N/A	

Age = age at admission to study, N/A = not applicable, SD = standard deviation; VISA = V (vision, identify the presence of dysthyroid optic neuropathy), I (orbital soft tissue inflammation or congestion), S (strabismus, motility restriction), and A (appearance, exposure); NAION = non-arteritic anterior ischemic optic neuropathy; TAO = thyroid-associated ophthalmopathy

outlier with a VISA score and overall mean RNFL thickness both over 2 standard deviations from the mean. In both analyses the overall correlation might be attributed to the correlation between the VISA and the RNFL in the superior quadrant (R = 0.788 and 0.649 with and without excluding the suspected outlier, respectively).

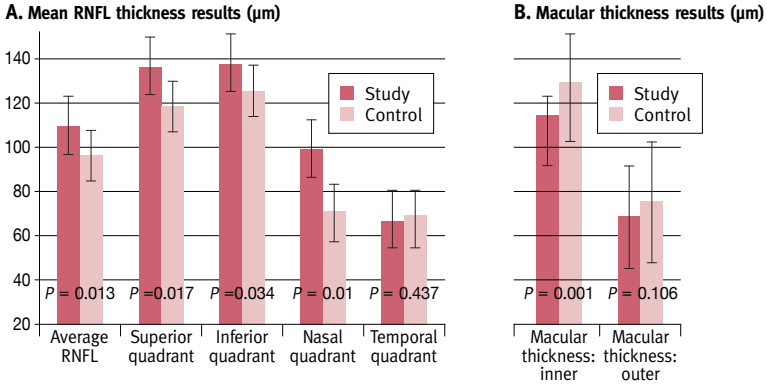
In trying to evaluate the nature of the correlation between the two parameters, we found consistently that the Pearson correlation was stronger and more significant than the Spearman correlation, implying a linear correlation between the VISA score and RNFL thickness [Figures 2A and 2B]. There was no correlation between the severity of the orbital disease as determined by VISA classification and the RNFL thickness in the temporal, inferior and nasal quadrants. A correlation also was not found between the severity of the orbital disease and the inner macular ring and the outer macular ring.

We used the Pearson product-moment correlation coefficient to check the correlation between the thickness of the inner macular ring and the following parameters in both the study and the control groups: mean RNFL thickness, thickness of the RNFL in each of the quadrants, and outer macular ring thickness. There was a significant correlation ($P < 0.05$) between the thickness of the inner macular ring and each of these parameters in the control group, but the only comparable correlation in the study group was between the inner macular ring and the outer macular ring thicknesses [Table 2].

DISCUSSION

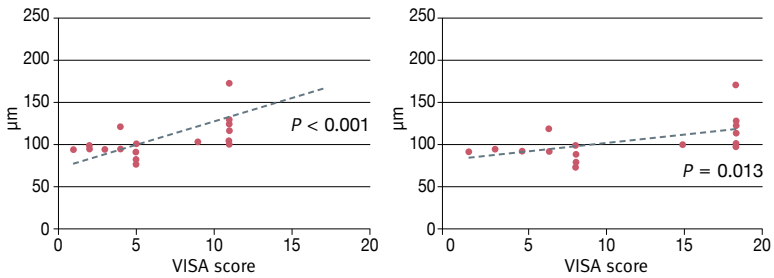
Our study showed that the eyes of TAO patients had a significant thickening in the superior, inferior and nasal quadrants of the peripapillary RNFL and a significant thinning of the inner macula compared to the eyes of the healthy control group. We also demonstrated a trend of thinning of the outer macula and a significant correlation between the severity of

Figure 1. [A] Comparison of the mean RNFL thicknesses (in µm) in the superior, inferior, nasal and temporal quadrants (Q) in 41 eyes of patients with TAO and 73 eyes of healthy subjects. **[B]** Comparison of the thickness (in µm) of the inner and the outer macular rings in 40 eyes of patients with TAO and 63 eyes of healthy subjects



RNFL= retinal nerve fiber layer, TAO = thyroid-associated ophthalmopathy

Figure 2. [A] Trend line showing the correlation between disease severity, as determined by the VISA classification, and the mean RNFL thickness. **[B]** The same trend line as in 2A, after excluding an outlier with a VISA score and mean RNFL thickness over 2 standard deviations from the mean



RNFL= retinal nerve fiber layer; VISA = V (vision, identify the presence of dysthyroid optic neuropathy), I (orbital soft tissue inflammation or congestion), S (strabismus, motility restriction), and A (appearance, exposure)

Table 2. Results of RNFL and macula thicknesses in study and control groups

	Study group (N=21)	Control group (N=41)	*P* value
No. of eyes checked for RNFL thickness	41	73	
No. of eyes checked for macular thickness	40	63	
Overall mean RNFL thickness (µm)	110.06 ± 33.3	96.25 ± 9.42	0.013
Superior quadrant RNFL thickness (µm)	136.70 ± 45.73	118.47 ± 14.25	0.017
Inferior quadrant RNFL thickness (µm)	137.95 ± 35.03	125.52 ± 13.55	0.034
Nasal quadrant RNFL thickness (µm)	99.38 ± 65.85	71.91 ± 13.95	0.010
Temporal quadrant RNFL thickness (µm)	67.47 ± 13.88	69.1 ± 12.67	0.437
Inner macular ring thickness (µm)	270.40 ± 17.27	281.79 ± 15.20	0.001
Outer macular ring thickness (µm)	236.31 ± 14.78	241.42 ± 15.93	0.106

Values are mean ± standard deviation
RNFL = retinal nerve fiber layer

TAO and the mean RNFL thickness. By providing those objective measures of retinal damage, OCT may enhance our understanding of the mechanisms involved in TAO. To the best of our knowledge, no study to date has demonstrated changes in the retina associated with TAO.

There are a few possible explanations for the significant difference we found in the thicknesses of the RNFL and the inner macular ring between TAO and healthy eyes. Previous studies have shown that there is thinning of the RNFL in compressive optic neuropathy [11,12], as well as in patients with intracranial tumors that compress the optic chiasm and in patients with orbital and optic nerve tumors. Our findings of a thickening of the peripapillary RNFL point to the unlikelihood of a pressure-based etiology.

Another possible etiology is an inflammatory process. In order to identify clinical features leading to a diagnosis of optic neuropathy, McKeag and colleagues [13] found that 56% of eyes with definite optic neuropathy had disk edema and concluded that disk edema was a specific indicator of optic neuropathy. Their findings are in line with the thickening of the peripapillary RNFL seen in our TAO patients. The disk swelling may be secondary to the inflammation and edema that coexist in the orbit as a result of the thyroid disease. It is also possible that the compressive mechanical and the inflammatory mechanisms coexist, in which case the combination of optic disk atrophy with axon loss together with optic disk inflammation and swelling would result in a normal appearance of the optic disk on funduscopy.

The reason for the discrepancies between the findings of Wei et al. [6] and Forte et al. [7] and our observations is not completely clear but may be due to different methods for measuring optic nerve functioning and to different study populations. Wei and colleagues [6] used electrophysiological indices and we used the clinical VISA score, while Forte and co-authors [7] evaluated patients with Graves' ophthalmopathy and ocular hypertension and ours were TAO patients. Another possible explanation for these discrepancies may lie in the natural history of the disease: patients in different stages of TAO may display a wide range of findings.

The lack of any significant thinning of the RNFL in the temporal quadrant in eyes of TAO patients can be explained by the fact that the temporal retinal ring is usually the thinnest, making the difference between healthy and affected eyes smaller and therefore more difficult to detect.

The macular thinning in our TAO eyes may be secondary to mechanical compression on the retina by orbital contents, but we could not find any studies that address macular thinning in compressive orbital neuropathies. Similar macular thinning can be found in glaucomatous eyes, where it is well correlated with RNFL loss [14], and as a predominant feature in patients with autoimmune retinopathy due to damage caused by anti-retinal antibodies [15]. Because TAO has an immunologic base, we believe there is a need for further studies to evaluate the presence of auto-anti-retinal antibodies in TAO patients.

Another possible mechanism for macular thinning may be the decrease in blood flow to the retina of TAO patients. Fernandez-Buenaga et al. [16] found macular thinning in the eyes of patients with non-arteritic anterior ischemic optic neuropathy (NAION), which they attributed to the ischemic damage in the macula-papillary bundle that occurs in NAION. The decrease in blood flow in TAO disease may contribute to the macular thinning via vein congestion or arterial stenosis.

Our study has several limitations, the main one being its retrospective nature, which precludes controlling for confounding parameters. Another possible limitation is the relatively small number of patients, although our results reached a level of significance. Had our study been larger, subtle differences and correlations may have been found. Also, our subjects and controls were not age-matched, although the overall populations were fairly close in age. Finally, we used the time-domain rather than spectral-domain OCT, and the latter may have provided better visualization of the structural changes in TAO patients and segmentation of the retinal layers. Further studies are required to validate our findings and arrive at conclusions regarding the contribution of OCT at the different stages of TAO.

CONCLUSIONS

The findings of this study suggest that the retina is involved in TAO, probably as early as the subclinical stage. OCT may enable an early and accurate identification of RNFL thickening and macular thinning in TAO patients, as well as provide information about the severity of the orbital component valuable for both diagnosis and follow-up. Early diagnosis of optic neuropathy will enable timely evaluation and treatment, and help prevent or retard the development of more serious consequences.

Correspondence
Dr. S. Blum Meirovitch
Goldschleger Eye Institute, Sheba Medical Center, Tel Hashomer 5265601, Israel
Phone: (972-3) 530-2876
email: sharon.blum@gmail.com

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Capsule

Starving the pathogen

Actively killing pathogens is an important function of the immune response; equally important is limiting nutrient availability to the pathogen, a process known as nutritional immunity. Interleukin-22 (IL-22) plays an essential role in the resolution of infections at epithelial barrier sites, including the skin, lungs and intestines. Using a systemic model of *Citrobacter rodentium* infection, Sakamoto et al. uncovered an unexpected role for IL-22 in limiting availability of iron to the pathogen by promoting increased production of heme scavengers from the liver. Thus, beyond barrier immunity, IL-22 plays an additional role in regulating nutritional immunity in systemic bacterial infections.

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Eitan Israeli

Capsule

Ebola Superspreaders are local and disproportionate

Certain individuals, known as superspreaders, disproportionately infect more people with disease-causing organisms than the average infectious case. Lau et al. identified key drivers of Ebola virus (EBOV) superspreading during the 2014 West Africa outbreak. Unexpectedly, secondary cases largely did not transmit tertiary cases; thus, epidemic growth was fueled and sustained by a few superspreaders, and transmission occurred locally, within 2.5 km of the source. Community-based EBOV cases progressed more rapidly than did those identified in clinical care settings. The most infectious age groups tended to be the young or people over 45 years of age, which may reflect social structure, such as the intimacy of care needs or immunological factors. This work helps to identify the most vulnerable groups and provide parameters for control efforts in future outbreaks of EBOV.

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Eitan Israeli

Capsule

Influenz-ing IFN responses in dendritic cells

Seasonal influenza vaccines have been produced and marketed for decades, but they are not always protective. Athale and co-authors tested a trivalent vaccine against the monovalent vaccine made by the same manufacturer. They looked at the ability to activate human dendritic cell subsets, which are crucial for launching adaptive immune responses. Both vaccines could activate plasmacytoid dendritic cells, but only the trivalent vaccine could induce antiviral interferon (IFN) responses in other types of dendritic cells. Moreover, people immunized with the monovalent vaccine did not show early IFN responses in the blood, but those immunized by trivalent vaccination did. These results may help to explain vaccine underperformance that cannot be attributed to antigenic mismatch.

Sci Transl Med 2017; 9: eaaf9194

Eitan Israeli