MEDICAL SCIENCE MONITOR

CLINICAL RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2019; 25: 1024-1031
DOI: 10.12659/MSM.912310

Received: 2018.07.26
Accepted: 2018.11.01
Published: 2019.02.06

Altered Corneal Nerves in Chinese Thyroid-Associated Ophthalmopathy Patients Observed by *In Vivo* Confocal Microscopy

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Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Source of support: This study was supported by the National Natural Science Foundation of China (Grant No. 81600765) and the Foundation of Shanghai Municipal Commission of Health and Family Planning (Project No. 201640120)

Background: Thyroid-associated ophthalmopathy (TAO) is a common endocrine autoimmune disease. The present study explored corneal nerve changes in TAO patients.

Material/Methods: Thirty-eight Chinese TAO patients and 20 healthy individuals were included in the study. Central corneal subbasal nerve density and morphology were evaluated with *in vivo* laser scanning confocal microscopy and quantified using automated CCmetrics software.

Results: The values of the central corneal subbasal nerve plexus parameters of both active and inactive TAO patients were significantly decreased compared with those of controls, including corneal nerve fiber density (CNFD) ($P<0.001$ for both), corneal nerve branch density (CNBD) ($P<0.001$ for both), corneal nerve fiber length (CNFL) ($P<0.001$ for both), corneal nerve fiber total branch density (CTBD) ($P<0.001$ for both), corneal nerve fiber area (CNFA) ($P<0.001$ for both), corneal nerve fiber width (CNFW) ($P=0.046$, $P=0.027$, respectively), and corneal nerve fiber fractal dimension (ACNFrD) ($P<0.001$ for both). In addition, CNFD and ACNFrD values were significantly lower in the active TAO patients compared with those in the inactive TAO patients ($P=0.020$, $P=0.002$, respectively). There were significant correlations between CNFD, CNBD, CNFL, CTBD, CNFA, and ACNFrD and the ocular surface parameters and activity assessment items.

Conclusions: Abnormal corneal subbasal nerves were observed in both active and inactive Chinese TAO patients, suggesting that nerve degeneration is associated with the disease. However, the exact underlying mechanisms remain to be elucidated.

MeSH Keywords: Cornea • Graves Ophthalmopathy • Microscopy, Confocal • Nerve Fibers

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/912310

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Background

Thyroid-associated ophthalmopathy (TAO) is a long-lasting, chronic orbital disease, which is associated with a combination of environmental, genetic, and immunological factors [1–3]. Ocular surface impairment in TAO, such as conjunctival hyperemia and chemosis, dry eye, superficial punctate keratopathy, exposure keratopathy, and corneal ulcer, is common [4–6]. The cornea is innervated by nerve fibers from the ophthalmic division of the trigeminal nerve [7], which are responsible for the sensation of touch, pain, and temperature, and play important roles in the blink reflex, corneal epithelial integrity, and tear production and secretion [7–9]. In addition, neurotrophic factors released from the corneal nerves assist with maintenance of the healthy cornea and modulate wound repair [8]. Corneal nerve dysfunction can lead to a series of ocular surface problems, such as dry eye, persistent corneal epithelial defects, and neurotrophic keratopathy, and it is also a frequent feature of diseases that affect the cornea [8].

In vivo confocal microscopy (IVCM) has become an indispensable tool in the study of corneal physiology and disease, allowing direct noninvasive visualization of small fiber corneal nerve microstructures [10]. In our study, we assessed the structural changes of corneal subbasal nerves in TAO patients via *in vivo* laser scanning confocal microscopy (LSCM).

Material and Methods

Subjects

Thirty-eight TAO patients and 20 healthy individuals were enrolled from the Department of Ophthalmology, Changzheng Hospital. All the participants were Chinese. The diagnosis of TAO was based on the Bartley criteria [11]. TAO activity was classified according to the clinical activity score (CAS), which consists of 7 items, including spontaneous retrobulbar pain, pain on attempted upward or downward gaze, redness of eyelids, redness of conjunctiva, swelling of caruncle or plica, swelling of eyelids, and swelling of conjunctiva (chemosis) [12]. For each sign that is present, 1 point is given. A CAS ≥3/7 is indicative of active TAO, and a CAS ≤2/7 is indicative of inactive TAO [12]. TAO severity was categorized as mild, moderate-to-severe, or sight-threatening (very severe), according to the standardized criteria of the European Group on Graves' Orbitopathy (EUGOGO) [12].

All the patients were hyperthyroid upon initial diagnosis of TAO and were treated by antithyroid drugs. Their thyroid functions were within normal range after the therapy. Patients were excluded if they had received radioactive iodine therapy or thyroidectomy, had suffered from other ocular disorders or systemic autoimmune diseases prior to the onset of TAO, had received either systemic or local treatment that might affect ocular surface parameters, wore contact lenses, or had undergone ophthalmic surgery. The normal subjects were recruited during routine medical care. Those who wore contact lenses, used systemic drugs or topical eye drops, or had a history of ocular or systemic diseases, ocular trauma, or surgery were also excluded.

The study was approved by the Ethics Committee of Changzheng Hospital, which is affiliated to the Second Military Medical University; the study also adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from each participant.

Clinical evaluation

The medical history of each participant was recorded. All individuals received a full ophthalmic examination of both eyes, which included visual acuity, intraocular pressure, proptosis, lagophthalmos, slit-lamp, and fundus examination. The CAS and severity of TAO were evaluated for each subject. Ocular surface damage was assessed using the Ocular Surface Disease Index (OSDI) symptom questionnaire, tear break-up time (BUT), fluorescein staining, and Schirmer test I. Corneal sensitivity was evaluated with a Cochet-Bonnet nylon thread esthesiometer. All ophthalmic examinations were performed by the same researcher. The serum levels of the free tri-iodothyronine (FT3), free thyroxine (FT4), and thyroid-stimulating hormone (TSH) of each subject were examined. In addition, all of the participants were evaluated for their smoking habits.

Confocal microscopy

Bilateral IVCM of the cornea was performed on all patients with a Heidelberg Retina Tomograph (HRT3) equipped with a Rostock Corneal Module (RCM) (Heidelberg Engineering GmbH, Heidelberg, Germany). This microscope uses a 670-nm diode laser as a light source and results in an image dimension of 400×400 μm^2. Both eyes of the participants were anesthetized with 1 drop of topical anesthetic solution (0.5% proxymetacaine hydrochloride, Alcaine; Alcon Laboratories, Fort Worth, TX). Carbomer gel (Vidisic; Bausch & Lomb, Rochester, NY) was used as a coupling medium between the applanation lens and the cornea. The patients were instructed to fixate on a distant target before examination. IVCM was performed within the central 2-mm area of the cornea. The examiner could monitor the live imaging of the cornea using a CCD camera attached to the microscope to determine the location of examination. The central cornea means the reflex of the laser beam on the cornea was in the center of the pupil. *In vivo* LSCM was performed by the same expert examiner using the sequence scan mode at the Eye and ENT Hospital of Fudan University.

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Table 1. Demographic results and clinical profiles of TAO patients and healthy subjects.

	Active TAO (N=14)	Inactive TAO (N=24)	Control (N=20)	*P*
Gender (Male/Female)	5/9	5/19	7/13	0.491*
Age (years)	43.50±5.71	40.00±7.64	44.10±7.28	0.132**
Time since symptom onset (month)	15.64±13.01	57.79±42.82	N/A	<0.001***
Clinical activity score	5.71±1.27	1.17±0.76	0	<0.001**
Severity of TAO (mild/moderate-to-severe/very severe)	0/14/0	5/19/0	N/A	0.137*
FT3 (pmol/L)	5.28±1.89	4.59±0.65	4.51±0.49	0.086**
FT4 (pmol/L)	17.09±5.02	15.25±3.74	15.31±1.18	0.248**
TSH (mU/L)	3.64±6.34	2.44±2.47	2.41±0.94	0.538**
Smoking habits (smoker/ex-smoker/nonsmoker)	3/2/9	6/2/16	7/1/12	0.817*

N/A – not applicable for this group; FT3 – free triiodothyronine; FT4 – free thyroxine; TSH – thyroid stimulating hormone. * Fisher's exact test; ** – ANOVA; *** – *t* test.

Image analysis

For each patient, at least 300 good-quality images of all corneal layers were obtained.

The subbasal nerve plexus is located in the subepithelial area, between the basal epithelial and Bowman layers. The approach for image selection was as follows. First, all the images of the subbasal nerve plexus were selected based on the best focus and good contrast. Then, 8 images that did not overlap by more than 20% were chosen for analysis in a masked fashion by the same observer [13]. These images were analyzed using a fully automated nerve fiber image analysis program (ACCMetrics; M.A. Dabbah, Imaging Science and Biomedical Engineering, Manchester, UK) [14] to quantify the following parameters:

1. corneal nerve fiber density (CNFD): the number of nerve fibers per mm^2, 2. corneal nerve branch density (CNBD): the number of branch points on the main nerve fibers per mm^2, 3. corneal nerve fiber length (CNFL): the total length of nerve per mm^2, 4. corneal nerve fiber total branch density (CTBD): the total number of branch points per mm^2, 5. corneal nerve fiber area (CNFA): the total nerve fiber area per mm^2, 6. corneal nerve fiber width (CNFW): the average nerve fiber width per mm^2, and 7. corneal nerve fiber fractal dimension (ACNFrD): the fractal dimension measurement is fully automated and consists of a nerve fiber detection step based on a machine-learning method [15]. Then, the ACNFrD is calculated using a box-counting method based on the detected nerve fibers from the corneal confocal microscopy images, as described previously [16]. The ACNFrD is able to measure the spatial loss of nerve fibers. A high ACNFrD value likely corresponds to a healthy subject, and a lower value may reflect abnormality [17].

Statistical analysis

TAO is usually bilateral, but it can be asymmetric or unilateral. Specific treatments for TAO vary depending on the severity of the disease, which is based on the clinical features in the worse eye [18]. Thus, in order to evaluate the ocular surface status and corneal innervation in TAO patients and avoid underestimation, the eye with a higher fluorescein staining score for each individual was selected for analysis, as previously described by Villani et al. [5]. In the event that both eyes had equal fluorescein staining scores, the sequential discriminating criteria were a lower Schirmer test score and lower BUT score [5]. Data distribution and homogeneity were analyzed. Normally distributed variables were expressed as the mean ± standard deviation, and a t test was used to compare the mean values between the active and inactive TAO groups; a one-way ANOVA with the LSD post hoc test or Tamhane's T2 test were used to compare the mean values of the control group and the active and inactive TAO groups. Skewed variables were expressed as median values and percentiles (*Q1, Q3*), and the Mann-Whitney *U* nonparametric test was applied. Fisher's exact test was applied to analyze the categorical variables. Correlations between variables were analyzed using Spearman's rank correlation test. A *P*-value of less than 0.05 was considered statistically significant (SPSS 19.0; IBM, Chicago, IL).

Results

Demographic data and clinical features

The demographic data and clinical features of all the participants are presented in Table 1. There were no significant differences between the active TAO, inactive TAO, and control

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Table 2. Ocular surface parameters and activity assessment of TAO patients and control subjects.

	Active TAO (N=14)	Inactive TAO (N=24)	Control (N=20)	*P*
Proptosis (mm)	21.07±2.46	19.69±2.13	15.03±0.73	<0.001*
Lagophthalmos (mm)	1.25 (0, 3.5)	0 (0, 1.75)	0 (0, 0)	<0.001**
Palpebral fissure height (mm)	11.00±2.60	10.92±1.86	7.65±0.99	<0.001*
Redness of eyelids (%)	100% (14/14)	4.2% (1/24)	0% (0/20)	<0.001***
Swelling of eyelids (%)	100% (14/14)	75.0% (18/24)	0% (0/20)	0.067***
Redness of conjunctiva (%)	100% (14/14)	33.3% (8/24)	0% (0/20)	<0.001***
Swelling of conjunctiva (%)	100% (14/14)	0% (0/24)	0% (0/20)	<0.001***
Swelling of caruncle (%)	78.6% (11/14)	0% (0/24)	0% (0/20)	<0.001***
OSDI (score)	52.09 (46.36, 56.25)	25.00 (20.83, 39.06)	0 (0, 1.56)	<0.001*
BUT (seconds)	5.17±2.22	6.45±2.95	11.62±1.67	<0.001*
Fluorescein staining (score)	3.71±4.14	1.83±2.08	0	<0.001*
Schirmer test (mm)	5.32±2.11	6.27±2.93	12.65±1.95	<0.001*
Corneal sensitivity	5.33±0.66	5.46±0.47	5.80±0.32	0.014*

* ANOVA; ** active TAO *vs.* inactive TAO, Mann-Whitney *U* nonparametric test; *** active TAO *vs.* inactive TAO, Fisher's exact test.

Table 3. Confocal microscopy data of TAO patients and control subjects.

	Active TAO (N=14)	Inactive TAO (N=24)	Control (N=20)	*P*#
Corneal nerve fiber density (n/mm^2)	12.3±4.7	17.2±6.2	28.4±6.7	<0.001
Corneal nerve branch density (n/mm^2)	13.2±8.6	20.8±13.1	41.9±16.0	<0.001
Corneal nerve fiber length (mm/mm^2)	10.4±3.0	12.3±3.1	17.6±3.6	<0.001
Corneal nerve fiber total branch density (n/mm^2)	27.6±11.8	36.9±17.0	56.5±19.8	<0.001
Corneal nerve fiber area (mm^2/mm^2)	0.005±0.001	0.005±0.002	0.007±0.002	<0.001
Corneal nerve fiber width (mm/mm^2)	0.02±0.001	0.02±0.001	0.02±0.001	0.050
Corneal nerve fiber fractal dimension	1.4±0.04	1.5±0.04	1.5±0.03	<0.001

ANOVA.

groups with regard to sex, age, levels of FT3, FT4, and TSH, or smoking habits. CAS was significantly higher in active TAO patients compared to inactive TAO patients. The severity of TAO did not differ between the active and inactive TAO groups.

Ocular surface parameters

The ocular surface parameters of TAO patients and control subjects are summarized in Table 2. All TAO patients had dry eye, as evidenced by the significantly higher OSDI and fluorescein staining scores and the lower BUT and Schirmer test values compared with the normal controls. Corneal sensitivity was decreased in inactive TAO patients compared with that in control subjects (*P*=0.022) but did not differ between the active TAO and control groups.

Activity assessment of TAO

The presence of eyelid redness, conjunctiva redness, conjunctiva swelling, and caruncle swelling was significantly higher in active TAO patients compared to inactive TAO patients, respectively, while the presence of eyelid swelling did not differ between the 2 groups (Table 2).

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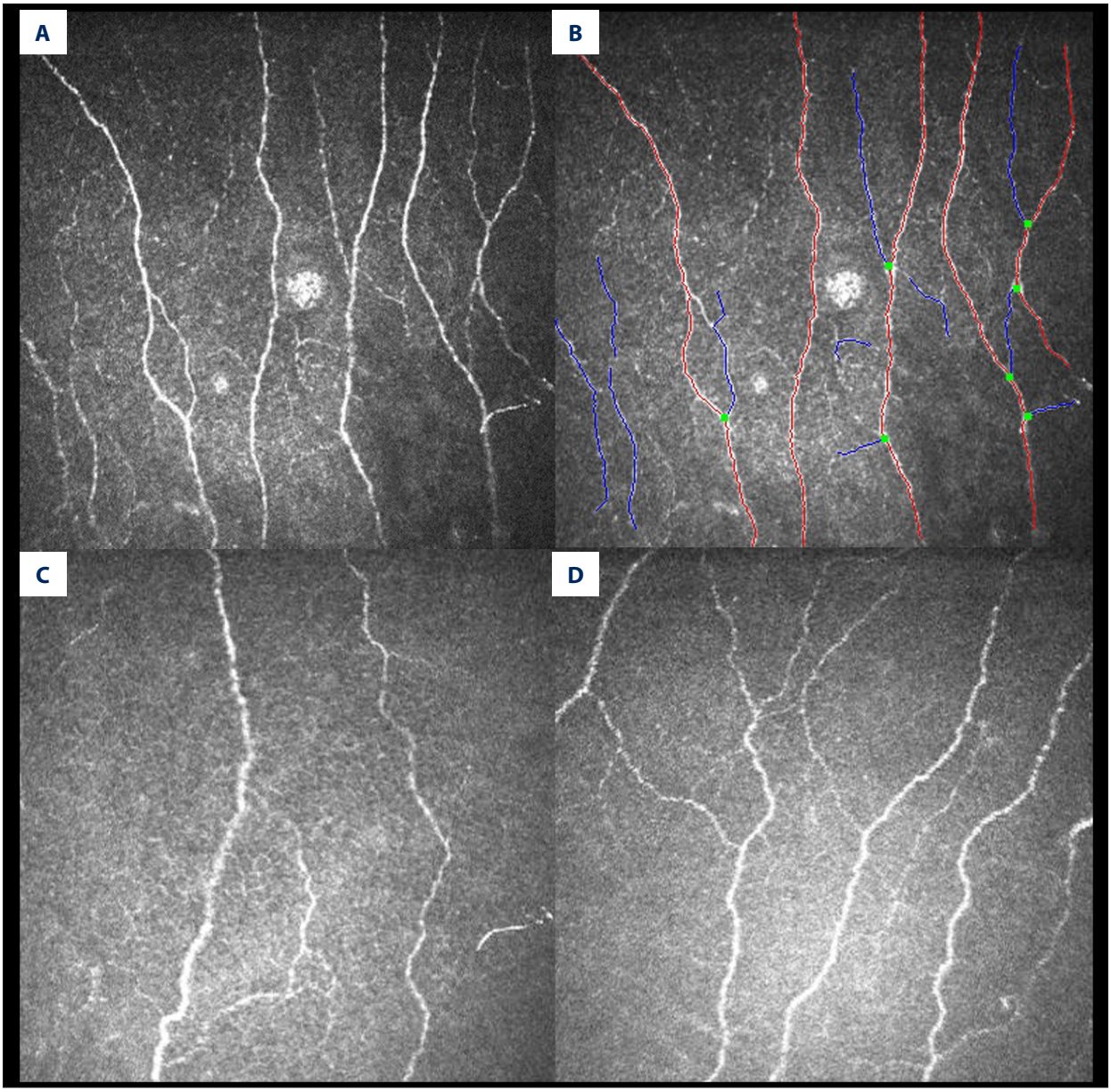

Figure 1. Representative confocal microscopic images of the corneal subbasal nerve plexus in TAO patients and control subjects. (**A**) Corneal subbasal nerve plexus of a normal subject and (**B**) the same confocal microscopic image analyzed by the automated CCmetrics software. The quantitative analysis results were corneal nerve fiber density (CNFD)=31.2 n/mm^2, corneal nerve branch density (CNBD)=43.7 n/mm^2, corneal nerve fiber length (CNFL)=17.0 mm/mm^2, corneal nerve fiber total branch density (CTBD)=50.0 n/mm^2, corneal nerve fiber area (CNFA)=0.006 mm^2/mm^2, corneal nerve fiber width (CNFW)=0.02 mm/mm^2, and corneal nerve fiber fractal dimension (ACNFrD)=1.5. (**C**) Corneal subbasal nerve plexus of an active TAO patient, and the quantitative analysis results were CNFD=18.7 n/mm^2, CNBD=43.7 n/mm^2, CNFL=13.3 mm/mm^2, CTBD=62.5 n/mm^2, CNFA=0.007 mm^2/mm^2, CNFW=0.02 mm/mm^2, and ACNFrD=1.5. (**D**) Corneal subbasal nerve plexus of an inactive TAO patient, and the quantitative analysis results were CNFD=18.7 n/mm^2, CNBD=12.5 n/mm^2, CNFL=7.6 mm/mm^2, CTBD=18.7 n/mm^2, CNFA=0.002 mm^2/mm^2, CNFW=0.03 mm/mm^2, and ACNFrD=1.4. Note the decreased number of nerves and increased tortuosity in active TAO (**C**) and inactive TAO patients (**D**).

Confocal microscopy data

Table 3 shows the confocal microscopy results for the various groups. The subbasal nerve plexus parameter values in both the active and inactive TAO patients were significantly decreased compared to the controls (Figure 1), including CNFD (P<0.001 for both), CNBD (P<0.001 for both), CNFL (P<0.001 for both), CTBD (P<0.001 for both), CNFA (P<0.001 for both),

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Table 4. Correlations between the confocal microscopy data and clinical data.

		CNFD	CNBD	CNFL	CTBD	CNFA	CNFW	ACNFrD
Severity of TAO	r	–0.046	0.050	–0.053	–0.142	–0.106	–0.202	–0.018
	P	0.783	0.767	0.751	0.395	0.525	0.223	0.916
Redness of eyelids	r	–0.563	–0.498	–0.469	–0.449	–0.347	0.171	–0.556
	P	**<0.001**	**<0.001**	**<0.001**	**0.001**	**0.008**	0.201	**<0.001**
Swelling of eyelids	r	–0.639	–0.582	–0.598	–0.548	–0.505	0.220	–0.623
	P	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.098	**<0.001**
Redness of conjunctiva	r	–0.462	–0.412	–0.416	–0.357	–0.350	0.058	–0.475
	P	**<0.001**	**0.001**	**0.001**	**0.006**	**0.007**	0.663	**<0.001**
Swelling of conjunctiva	r	–0.513	–0.472	–0.436	–0.409	–0.354	0.108	–0.525
	P	**<0.001**	**<0.001**	**0.001**	**0.001**	**0.006**	0.418	**<0.001**
Swelling of caruncle	r	–0.500	–0.514	–0.338	–0.406	–0.225	0.041	–0.456
	P	**<0.001**	**<0.001**	**0.010**	**0.002**	**0.090**	0.761	**<0.001**
OSDI	r	–0.601	–0.526	–0.571	–0.440	–0.402	0.196	–0.586
	P	**<0.001**	**<0.001**	**<0.001**	**0.001**	**0.002**	0.141	**<0.001**
BUT	r	0.434	0.367	0.382	0.245	0.194	–0.022	0.440
	P	**0.001**	**0.005**	**0.003**	**0.064**	**0.145**	0.870	**0.001**
Fluorescein staining score	r	–0.292	–0.259	–0.249	–0.190	–0.144	0.181	–0.216
	P	**0.026**	0.050	0.060	0.154	0.279	0.174	0.103
Schirmer test score	r	0.598	0.547	0.545	0.478	0.407	–0.245	0.595
	P	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.002**	0.064	**<0.001**
Corneal sensitivity	r	0.272	0.315	0.258	0.254	0.188	–0.202	0.217
	P	**0.039**	**0.016**	0.051	0.055	0.157	0.129	0.102

Bold values indicate statistical significance ($P<0.05$).

CNFW ($P=0.046$, $P=0.027$, respectively), and ACNFrD ($P<0.001$ for both). In addition, CNFD and ACNFrD in the active TAO patients were significantly lower compared to those in the inactive TAO patients ($P=0.020$, $P=0.002$, respectively). However, there were no statistically significant differences in CNBD, CNFL, CTBD, CNFA, and CNFW between the active and inactive TAO groups ($P=0.110$, $P=0.091$, $P=0.109$, $P=0.274$, and $P=0.942$, respectively).

Correlations

The correlations between the confocal microscopy data and the clinical data are shown in Table 4. CNFD and CNBD were significantly correlated with the redness of eyelids, swelling of eyelids, redness of conjunctiva, swelling of conjunctiva, swelling of caruncle, OSDI scores, BUT, Schirmer test scores, and corneal sensitivity, respectively. In addition, CNFD was inversely correlated with the corneal fluorescein staining score. CNFL and ACNFrD were significantly correlated with the redness of eyelids, swelling of eyelids, redness of conjunctiva, swelling of conjunctiva, swelling of caruncle, OSDI scores, BUT, and Schirmer test scores. CTBD was significantly correlated with redness of eyelids, swelling of eyelids, redness of conjunctiva, swelling of conjunctiva, swelling of caruncle, OSDI scores, and Schirmer test scores. CNFA was significantly correlated with redness of eyelids, swelling of eyelids, redness of conjunctiva, swelling of conjunctiva, OSDI scores, and Schirmer test scores. No significant correlation was found between the CNFW and clinical data.

Discussion

The ocular surface and lids, the lacrimal gland, and the interconnected reflex arcs constitute a functional unit [19]. In a physiological eye, the stimulation of afferent nerves produces

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lid closure and tear secretion. Malfunction of nerves in the cornea will disrupt homeostasis of the ocular surface, leading to dry eye and other ocular surface disorders [19].

TAO is an autoimmune inflammatory disease that can affect the cornea and lacrimal gland function [4–6]. However, studies assessing corneal innervation in TAO patients are scarce [5]. Villani et al. reported that the subbasal nerve number was significantly decreased in Graves orbitopathy patients compared to control subjects using slit-scanning confocal microscopy (SSCM) [5]. Indeed, 3 types of IVCM are available, including tandem-scanning confocal microscopy, SSCM, and LSCM, all of which represent the development of the field of confocal microscopy. In the present study, we applied *in vivo* LSCM to evaluate the corneal subbasal nerves in active and inactive TAO patients of Chinese ethnicity and normal subjects. The CNFD and CNBD in the control subjects were 28.4±6.7 n/mm^2 and 41.9±16.0 n/mm^2, respectively, and the total number of corneal subbasal nerves was 11.2 n/frame, which was approximately 2 times higher than the number reported by Villani et al. [5]. Indeed, subbasal nerve densities vary extensively depending on the type of IVCM [20], image selection criteria, and nerve tracing and analysis software used [21]. Here, to minimize the additional bias in image selection, 8 images not overlapping by more than 20% were used for analysis, according to the study of Vagenas et al. on the optimal image sample size for corneal nerve morphometry [13]. In addition, the fully automated nerve fiber image analysis program used in this study is a reliable and objective method for quantifying the corneal subbasal nerves that generates results with a high level of reproducibility and has been proven to agree very well with semiautomated and manual analysis [14, 22].

In comparison to normal subjects, TAO patients exhibited compromised corneal innervation and decreased corneal sensitivity. These results are consistent with those of Villani et al. [5]. In addition, the active and inactive TAO groups differed mainly in the CNFD and ACNFrD. ACNFrD, a novel metric of corneal nerve morphology, was proposed to measure the spatial loss of nerve fibers [17]. The study of Chen et al. demonstrated that ACNFrD is comparable to CNFD, CNBD, and CNFL in diagnosing patients with and without diabetic neuropathy [17]. The value of ACNFrD was significantly reduced in the active and inactive TAO patients compared to the control subjects, as well as in the active TAO patients compared to the inactive TAO patients, which provides additional evidence to indicate different neurodegenerative conditions in these active and inactive TAO patients.

However, the exact cause the corneal nerve changes in TAO remains unclear. First, dry eye in patients with TAO may be attributed to the corneal subbasal nerve changes in this study. Here, the density and length of the central corneal subbasal nerves were significantly correlated with the BUT, Schirmer test scores, and OSDI scores, indicating the severity of dry eye. Dry eye has been reported to be a type of neurotrophic keratopathy [23]. A decrease in subbasal nerve densities has been previously reported in various autoimmune types of dry eye, including dry eye with primary or secondary Sjogren's syndrome (secondary to rheumatoid arthritis) [23,24]. It was reported that corneal nerves were decreased in density in response to noxious mechanical, thermal, and chemical stimuli [25]. Tear hyperosmolarity, which typically presents in dry eye disease, as well as in Graves orbitopathy [26], may damage corneal nerve fibers. Similar results were found by Hirata et al., who reported that tear hyperosmolarity significantly decreased the physiological sensitivity and morphologic integrity of the corneal nerves [27]. Second, the ocular surface inflammation may also play a role in the loss of the subbasal nerve plexus. TAO patients, especially the active cases, can manifest ocular adnexa inflammation. Huang et al. reported that TNF-α concentration in tears was significantly higher in both active and inactive TAO compared with the controls [28]. We reported an increase in the density and maturation of corneal Langerhans cells in patients with TAO, which indicates an inflammatory process in the cornea [6]. In this study, CNFD, CNBD, CNFL, CTBD, CNFA, and ACNFrD were inversely correlated with the inflammation of the eyelid and conjunctiva. Kocabeyoglu et al. reported that inflammatory mechanisms may be responsible for decreased subbasal nerve density in newly diagnosed Graves' disease patients with no evidence of active thyroid eye disease [29]. Villani et al. reported that the reduced corneal nerve numbers, and increased nerve tortuosity and the number of beadlike formations in Graves' orbitopathy patients seem to be affected by inflammatory processes related to dry eye and systemic disease itself [5]. The nervous and immune systems communicate biochemically, and excessive inflammation may lead to a loss of corneal innervation [30]. Finally, increased lid retraction on the ocular surface during blinking due to lid friction, lid swelling, and proptosis in TAO patients is a kind of mechanical damage, which may cause cornea nerve dysfunction. However, these hypotheses must be confirmed.

One of the limitations of this study is not including Graves's disease patients without TAO. Further evaluations and comparisons of the corneal subbasal nerves by *in vivo* confocal microscopy in Graves' disease without TAO, Graves' disease with TAO, and in nonspecific dry eye are needed to confirm that the abnormal corneal subbasal nerves are due to TAO, dry eye, or both. Basic research is needed to explore the exact mechanisms responsible for the ocular surface changes in TAO. There are 2 more limitations of this study. One is the relatively small sample size, and the other is the automated nerve fiber image analysis software used in this study, which could not quantify the parameters representing the corneal nerve morphology, including corneal nerve tortuosity and reflectivity [5].

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Conclusions

We observed the density and morphological changes of corneal subbasal nerves in active and inactive Chinese TAO patients, although the underlying mechanisms remain to be elucidated and warrant further study.

Conflicts of interest

None.

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