Original Article

Ocular Surface Characteristics and Impression Cytology in Patients with Active versus Inactive Thyroid Eye Disease

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Abstract

Purpose: To compare the clinical findings, tear film function and impression cytology between patients with active and inactive Thyroid Eye Disease (TED).

Methods: A total of 56 patients with TED and 30 controls were recruited in this prospective observational cohort study. TED patients were divided into active TED and inactive TED types according to a seven-point modified formulation of the Clinical Activity Score (CAS). All participants underwent full eye examinations including Ocular Surface Disease Index (OSDI) score, tear film break-up time (TBUT), fluorescein staining and Schirmer I test. Thirty nine patients with thyroid-associated orbitopathy (TAO) received Nelson's grade with conjunctival impression cytology. Proptosis, palpebral fissure width and lagophthalmos were assessed.

Results: Ocular surface parameters including proptosis, palpebral fissure width and lagophthalmos did not differ between active and inactive TED patients (P>0.05). Both active and inactive TED patients obtained higher fluorescein staining scores, lower TBUT scores and significantly lower Schirmer test scores than those of controls (P<0.001 for all). Additionally, the TBUT score was significantly lower and the OSDI score significantly higher in the active TED group compared with those in the inactive TED group (P<0.001 for both). Impression cytology revealed a higher proportion of grade 2-3 changes in the active TED group compared with the inactive TED group(P<0.001).

Conclusion: Orbital inflammation in TED patients may lead to decreased tear film stability and ocular surface squamous metaplasia. (***Eye Science 2012; 27:64-68***)

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Thyroid eye disease (TED), also known as thyroid-associated ophthalmopathy, is the most common inflammatory orbital disease in adults[1], viewed as a multi-system autoimmune disease involving thyrocytes and orbital fibroblasts. Although only 3-5% of patients with TED have sight-threatening corneal ulceration or compressive optic neuropathy[2], many TED patients have symptoms of ocular surface discomforts, including excess tearing, gritty sensation, increased sensitivity to light, and foreign body sensation. Epidemic study revealed that a third of patients with TED have features of dry eye disease[3].

The etiology of ocular surface damage in TED patients is not fully understood, but is known to involve various factors. Upper eyelid retraction, increased proptosis, greater palpebral fissure width and increasing the tear evaporation, which are responsible for ocular surface damage[4-7]. Recent research found that inflammation may play a key role in ocular surface damage, and that lacrimal gland and tear fluid may be directly involved. A retrospective observational case study suggested that the TED was a potential cause of inflammatory ocular surface disease with dry eye symptomatology[8]. Oral steroid treatment can increase tear stability in active TED patients[9]. An in vivo confocal study revealed that the ocular surface inflammation in TED seems to be partially due to the autoimmune orbitopathy[10]. The present authors' previous study also found that clinical activity, proptosis, and palpebral fissure width were three significant risk factors causing ocular surface damage in TED patients[11]. However, the evi-

dence of orbital inflammation in the pathogenesis of ocular surface damage in TED was indirect, and few studies investigated the ocular surface characteristics in TED patients using impression cytology.

The present study compared tear film functions and ocular surface characteristics between active and inactive TED patients by performing full ophthalmologic examination, Schirmer I test, tear film breakup time test (TBUT), Ocular Surface Disease Index (OSDI) scores, and conjunctival epithelial impression cytology and comparison with healthy controls.

Patients and methods

Patients and controls: This case-control study included 56 TED patients and 30 age-gender matched normal controls. Written informed consents were obtained from all subjects in accordance with the guidelines of the Declaration of Helsinki. The diagnosis of TED was made on the basis of the Bartley's criteria[12]. Orbital inflammatory activity was evaluated using the guideline of seven-point modified formulation of clinical activity score (CAS), as previously described by Mourits[13]. TED patients were divided into active TED and inactive TED groups. The patients with CAS $\geqslant$3/7 were defined as active TED, and those with CAS<3/7 as inactive TED. The subjects who were previously treated with steroids or radiation therapy; suffered from ocular surface disorders such as dry eye syndrome, allergic ocular surface disease, or pterygium before the onset of TED; or received medications that might affect ocular surface parameters including rheumatoid arthritis, contact lens wearing, and topical anti-glaucoma drug use, were excluded from this study.

Clinical examination: Mechanical parameters of ocular surface (proptosis, palpebral fissure width, lagophthalmos) were measured in all TED patients. Ophthalmic examinations including visual acuity, silt lamp examination, fundus examination, TBUT, fluorescein staining (FL), and Schirmer I (SI) tests were taken in TED and control. TBUT was measured by instilling 5 μL of a 1% sodium fluorescein solution and calculating the average of three consecutive breakup times, manually determined with a stopwatch. FL was evaluated under cobalt blue illumination after fluorescein instillation. Staining scores were calculated following the National Eye Institute (NEI)/Industry Workshop scale[14]. A 5-minute SI test was performed with topical anesthesia with 0.5% proparacaine hydrochloride (Alcaine; Alcon). The filter paper strips were placed in the lateral fornices of the patients and controls. The length of wet papers was recorded. In order to quantify ocular surface symptoms in TED, visual analog symptom scores were calculated with an OSDI questionnaire consisting of 12 questions related to the effect of dry eye on vision-related functioning. This questionnaire score ranged from 0 to 100, including three sub jects: vision-related function (watching TV and reading), ocular symptoms (grittiness and blurred vision), and environmental triggers (low humidity and high wind)[15]. All ophthalmic examinations were performed bilaterally by the same researcher.

Impression cytology: The impression cytology specimens were obtained in 39 TED patients. After topical anesthesia with 0.5% proparacaine hydrochloride (Alcaine; Alcon), 0.2 mm porous Sartorius cellulose acetate filters were gently pressed on temporal bubal conjunctiva. The specimens were fixed with 95% ethyl alcohol and dyed with Papanicolaou stain. Specimens were evaluated with light microscopy and graded into four stages with Nelson grading system[16]. Specifically, Grade 0: small and round epithelial cells with strong intercellular con nections (with nucleocytoplasmic (n/c) ratio: 1:2); Grade 1: more large polygonal epithelial cells (with (n/c) ratio: 1:3); Grade 2: larger polygonal separate cells (with (n/c) ratio: 1–4:1–5); Grade 3: extremely large epithelial cells folded on themselves, where most cells lost their nucleus.

Statistical analysis

Data were expressed as the mean±SD. The mean value for both eyes was used for comparison of ocular surface parameters in TED. The student *t*-test was used to compare basic clinical data, and ocular surface parameters between active and inactive TAO after the normality of data was checked using the Shapiro-Wilk test. One-way ANOVA with the LSD post hoc test was used to compare TBUT, FL, SI tests among patients with active TED, inactive TED, and normal controls. Impression cytology data were compared by χ^2 test. $P<0.05$ was considered to

be statistically significant.

Results

Nineteen patients with active TED, 37 patients with inactive TED, and 30 gender-age matched normal controls were included. Clinical features between two eyes did not differ significantly. Hence, the mean value of the two eyes was used for statistical comparison. The demographic and clinical features in active and inactive TED were presented in Table 1. There were no significant differences in age, sex, or mechanical parameters of ocular surface between active and inactive TED patients. The mean OSDI scores in active TED patients were significantly higher compared with those in inactive TED patients (P=0.001).

Table 1 Comparison of disease profile between active and inactive TED patients

	Active TED	Inactive TED	*P*
Number	19	37	
Age	46.36±8.67	48.83±10.13	0.746 NS
Sex(M/F)	6/13	14/23	0.644 NS
OSDI scores	38.70±18.82	20.8±9.37	0.001
Proptosis	22.33±3.44	19.33±3.50	0.126 NS
Palpebral fissure width	12.33±2.67	12.11±3.33	0.867 NS
Lagophthalmos	1.33±1.80	1.25±1.96	0.922 NS

NS: not significant

The results of the ocular surface signs were presented in Table 2. Both active and inactive TED patients presented with higher FL scores, lower TBUT scores, and lower SI test scores compared with normal controls. TBUT scores obtained by patients with active TED were lower than those of inactive TED subjects.

Table 2 Comparison of ocular surface signs between TED patients and controls

	Active TED[1]	Inactive TED[2]	Control[3]	*P*
Number	19	37	30	–
Age (years)	46.36±8.67	48.83±10.13	48.57±8.82	0.866 NS
TBUT (s)	4.53±2.36	7.93±1.94	11.9±3.9	< 0.001[a]
St (mm)	6.86±11.42	6.20±4.02	12.1±3.8	< 0.001[b]
Fl scores	2.83±1.52	1.73±1.64	0.00±0.00	< 0.001[c]

a: 1 vs. 2: P<0.001; 1 vs. 3: P<0.001; 2 vs. 3: P<0.001
b: 1 vs. 2: P=0.736; 1 vs. 3: P<0.001; 2 vs. 3: P=0.002
c: 1 vs. 2: P=0.130; 1 vs. 3: P<0.001; 2 vs. 3: P<0.001
NS: not significant

The results of the impression cytology were shown in Table 3. Among 39 TED patients, the percentage of eyes with grade 2–3 squamous metaplasia on temporal bubal conjunctiva in the active TED group was demonstrated to be higher compared to the inactive TED group (P<0.001) (Figure 1), whereas the proportion of grade 0–1 squamous metaplasia in the inactive TED group was higher than in the active TED group (Figure 2).

Table 3 Comparison of impression cytology between active and inactive TED patients

Nelson grade	Active TED	Inactive TED	*P*
0–1(eyes)	12	42	<0.001
2–3(eyes)	18	6	

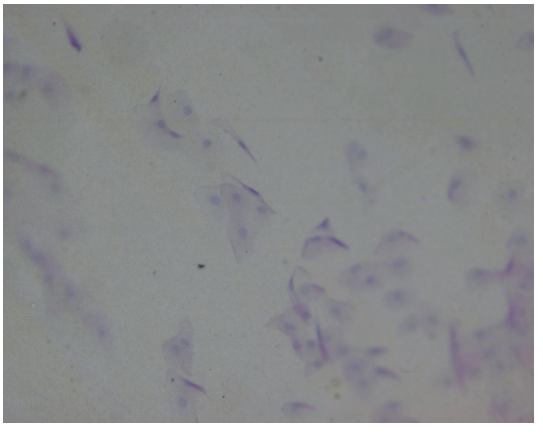

Figure 1 Grade 2–3 squamous metaplasia (×200, periodic acid Schiff staining).

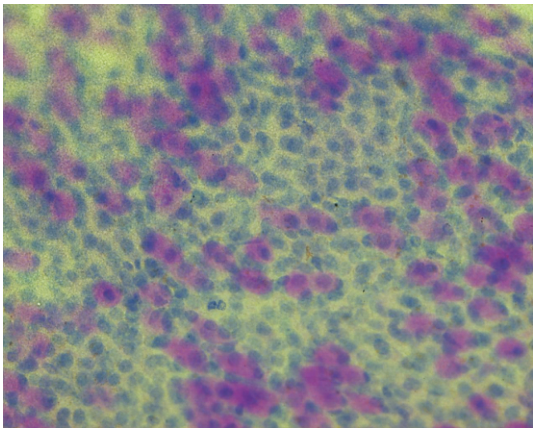

Figure 2 Grade 0–1 squamous metaplasia (×200, periodic acid Schiff staining)

Discussion

TED is an inflammatory disease occurring in the

orbital and adipose tissue, which has been reported to be associated with typical signs and symptoms of dry eye diseases. This study sought to investigate whether orbital inflammation had any effect on ocular surface in TED. The level of orbital inflammation in TED can be assessed by CAS. Grading the CAS of TED is fraught with difficulties; however, classifying patients into active/inactive TED categories is possible[17]. The present data indicate that ocular surface stability in TED with or without active phase were lower than normal controls. That is not surprising. Mechanical factors, including the increased proptosis and upper eyelid retraction, were mainly thought to be the causes of ocular surface damage in TED patients[4–7]. Furthermore, our results showed that BUT in active TED patients were significantly lower than inactive TED. This finding suggest that tear quality is affected in active TED, and this result was consistent with previous studies[4,6,9,18]. Yoon found that BUT significantly increased after steroid treatment in active TED patients, and contributed to both increased ocular surface and orbital inflammation[9]. In addition, we might contribute this result to the fact that active TED patients tend to suffer more ocular surface irregularities due to conjunctival chemosis and injection, which disturb tear film stability.

The data of impression cytology showed that there was a high incidence of grade 2–3 metaplastic changes in active TED patients compared with inactive TED patients. In other words, there were more goblet cell loss and squamous metaplasia of conjunctival epithelium in patients with active TED than in inactive TED. The pathogenesis of ocular surface squamous metaplasia epithelia can be ascribed to the loss of vascularization and scar formation[19]. Given the results that the mechanical parameters of ocular surface did not show statistically significant difference, the higher incidence of squamous metaplasia in active TED is probably because of the impact of orbital inflammation on ocular surface rather than the evaporation, viewed as beneficial evidence of the autoimmune orbit inflammation in the pathogenesis of ocular surface damage in TED.

Many studies provided evidence that ocular surface tissue may be the direct targets for autoantibodies in TED. Eckstein found that acinar cells from lacrimal glands in TED were positive for TSH-R, which is considered a target of autoimmunity shared by both thyroidal and extrathyroidal sites[18]. Imaging study of TED showed enlargement of lacrimal gland in some certain cases[20]. Pathologic examination of lacrimal gland in active TED showed interstitial edema and an increased number of lymphocytes[21]. Moncayo found a somatostatin analogue that exclusively binds to activated lymphocytes has been detected in lacrimal glands of TAO. Based on the above findings, the possible mechanism of ocular surface damage by orbital inflammation might be stated as follows: T cells sensitized to thyroid antigens recognize shared antigens on lacrimal acinar cell, which results in the production of hydrophilic glycosaminoglycans, causing lacrimal gland swelling and inhibit lacrimal gland function. Otherwise, Villani found keratocyte is involved directly in the ocular surface inflammation in active TED[10]. These results combined with the fact that many patients demonstrated episcleral and conjunctival hyperemia prior to typical TED signs indicated that the contribution of direct autoimmune targeting of the ocular surface cannot be excluded.

In conclusion, the findings of the present study demonstrate that orbital inflammation in TED patients may lead to decreased tear film stability and ocular surface squamous metaplasia. Therefore, anti-inflammatory agents such as topical steroids or topical CsA may be utilized to protect the ocular surface in TED patients. Previous reports have supported the benefit of anti-inflammation therapy in the treatment of this disorder[9,22]. Better-designed studies should be performed to investigate this subject.

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