Ocular surface changes in Graves’ ophthalmopathy

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Abstract
● Many patients with Graves’ ophthalmopathy (GO) suffer from dry eye syndrome (DES), and this is one of the most common reasons of eye discomfort in patients with GO. The prevalence of DES in patients with GO is significantly higher than normal subjects. The ocular surface changes involving changes in tears, cornea, conjunctiva and glands occur in GO patients. However, the mechanism of how DES occurs in GO still remains unclear. In this review, the ocular surface changes were illustrated and analyzed the reasons for high prevalence of DES in GO patients.
● KEYWORDS: Graves’ ophthalmopathy; dry eye syndrome; ocular surface
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INTRODUCTION
Graves’ ophthalmopathy (GO) usually occurs in patients suffering from Graves disease (GD) with hyperthyroidism or a history of hyperthyroidism[1]. In addition, it can also occur in patients with euthyroidism or hypothyroidism due to autoimmune thyroiditis. The ocular abnormalities are referred to GO, and its pathogenesis still remains unknown. The orbital tissue has cross-reactivity antigens with thyroid. They express the stimulating receptors targeting thyroid-stimulating hormone receptor (TSHR), and cause inflammatory reaction in the orbit[2]. Other antibodies that are targeted insulin-like growth factor-I receptor (IGF-1R) and interleukin (IL)-17 might also play important roles in the outbreak of GO[3-8]. Furthermore, the incidence of GO was the highest of orbital diseases[9], leading to symptoms such as proptosis, eyelid swelling, eyelid retraction, diplopia, photophobia, excessive tearing, vision loss, strabismus and eye movement disorder[10-11].

GO is devided into active GO and inactive GO according to the clinical activity scores (CAS)[12]. The presentation of each symptom mentioned below is considered as one point: moderate to severe eyelid erythema, moderate to severe eyelid swelling, conjunctival redness, chemosis, redness and swelling of lacrimal caruncle, spontaneous orbital pain and orbital pain evoked by gaze. Inactive GO is defined as those scoring 0-2 and active as scoring of 3-7[13]. Dry eye syndrome (DES) can prevail in any state or stage during the occurrence and development of GO.

DES was defined a multifactorial disease associated with tears and ocular surface by the Tear Film and Ocular Surface Society (TFOS) Dry Eye Workshop (DEWS) II. It causes discomfort, visual impairment as well as tear film instability, leading to damage of the ocular surface[14]. These symptoms also bother GO patients with DES and some of them might even progress to corneal injury and exposed keratitis[15-16].

For GO patients, proptosis and eyelid retraction might cause extra ocular exposure, increasing the evaporation of tears. The autoimmune and inflammatory responses might influence the composition of tears, leading to injury of the cornea and conjunctiva. GO also involves lacrimal glands and meibomian glands (MG). Therefore, the changes in tears, cornea, conjunctiva and glands in GO patients are considered as risk factors for DES[17].

EPIDEMIOLOGY
According to TFOS DEWS II, DES is one of the most common eye diseases, and the prevalence is about 5 to 50 percent. Based on the worldwide studies, the prevalence of DES in women is 1.33-1.74 times than that of men. In addition, age seems to be associated with positive symptoms of DES. The studies showed that the total prevalence of DES is about 14.4-24.4 percent in Asia, 14.5 percent in America, 18.4 percent in Spain, 20 percent in United Kingdom and 39.2 percent in France. Many studies indicated that the prevalence changes slightly in people under 49 (about 15-25 percent), which increases with age (about 25-45 percent)[18].
The annual incidence of GO in women is about 16 cases per 100 000 people and 3 cases per 100 000 in men[17]. Middle-aged women are the main victims of GO. However, there is no clear data with regard to prevalence in patients of different ages and genders with GO suffering from DES. Some studies have estimated that 65 to 85 percent of GO patients suffer from DES[19,20]. For middle-aged women, the prevalence of DES is undoubtedly higher in GO patients than normal subjects (<20 percent).

**OCULAR SURFACE CHANGES**

**Objective Changes**

**Tears**

**Tear breakup time and Schirmer’s test** The changes in tears play a significant role in the occurrence of DES in GO patients. There are two basic indexes to estimate the condition of tears: the tear breakup time (BUT) and the Schirmer’s t test (SIt). BUT is a test that assesses the stability of the tear film, while SIt levels are the amount of basic secretion of tears. Wei et al[25] have reported that the BUT (8.40±2.80s) and SIt (8.53±3.54 mm) in a GO group showed statistically significant differences from that of the control group (13.47±3.62s, P=0.002; 14.87±7.12 mm, P=0.009). This illustrated damage to both quality as well as quantity of tears in GO. Many other reports have shown similar conclusions[21-23].

**The tear film osmolarity** The past studies have suggested that the osmolarity of the tear film is often higher in GO than in inactive GO, considering their association with cytokines in tears and found several differences in active GO, inactive GO and healthy controls.

The studies conducted by Kishazi et al[27] and Ujhelyi et al[28] have revealed that some cytokines in tears such as IL-1β, IL-6, IL-8, IL-13 are higher in GO patients than healthy subjects. In addition, IL-1β, IL-6 and IL-8 are significantly higher in active GO than in inactive GO, considering their association with CAS[25]. Other cytokines, in spite of no direct evidence in proving their relation with CAS, such as IL-17, IL-2, IL-10, tumor necrosis factor-α (TNF-α), interferon-γ (INF-γ), IL-7, IL-15, IL-17, IL-12p70, IL-21 and RANTES showed higher expression in GO patients[29-31].

The spectrum of proteins has altered in the tears of GO patients. In the study conducted by Matheis et al[30], some proteins were downregulated when compared with normal subjects by antibody microarray: proline-rich protein 1 (PROL1, P<0.05), proline-rich protein 4 (PRP4, P<0.05), calgranulin A (S10A8, P=0.004) and transcription activator BRG1 (SMCA4, P=0.002), while some were upregulated: Midasin and POTE-ankyrin-domain family-member I (P<0.05). Aass et al[31] have reported a significant increase of lysozyme C (LYZ), lactritin (LACRT) and zinc-alpha-2 glycoprotein 1 (AZGP1) by enzyme-linked immunosorbent assay (ELISA). Song et al[31] have reported overexpression of CD40, CD40 ligand, glucocorticoid-induced tumor necrosis factor receptor (GITR), IL-12p70, IL-1β, IL-2, IL-21, IL-6, macrophage inflammatory protein-3α (MIP-3α) and TRANCE in the tears of GO using high-throughput microarray technology. In addition, the downregulation of granulocyte-macrophage colony stimulating factor (GM-CSF), IL-1 sRIL, and IL-13 was also reported.

Furthermore, Choi et al[34] have reported increased levels of oxidative stress markers, 8-hydroxy-2’-deoxyguanosine (8-OHdG; r=0.676, P<0.001) and malondialdehyde (MDA; r=0.506, P=0.002) in GO patients, especially active GO, and are determined by ELISA. Also, CAS showed significant correlation with the concentrations of these.

**Cornea**

**Staining** Because of extra ocular exposure and abnormal state of tears in GO, patients might undergo corneal epithelial injury and even exposed to keratitis.

Corneal fluorescein staining assesses the area and density of damage. It is often graded from 1-4 in every quadrant and the higher the total score is, the more severe the injury caused. Li et al[35] have reported that almost 1/3 of GO patients suffer from epithelial injury and the results demonstrated statistical differences between active GO and inactive GO. Similar conclusion has been drawn by many other studies[23,26,36-37].

**In vivo confocal microscopy** In vivo confocal microscopy is a device that is used to scan and collect images of each layer of cornea and conjunctiva. After examining the density and morphology of Langerhans cells (LCs) of the cornea in 40 GO patients and 20 normal controls (NC), Wu et al[21] have found that both active and inactive GO patients have significantly higher central LC densities as well as higher central LC morphology values as compared to NC. In addition, the central LC density and central LC morphology value showed statistical correlations with CAS, ocular surface disease...
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index score and Schirmer test scores. According to a recent study conducted by Wu et al\textsuperscript{[21]}, the values of central corneal subbasal nerve plexus parameters in patients with active and inactive GO were significantly lower than the control group, including corneal nerve fiber density (CNFD), corneal nerve fiber length (CNFL), corneal nerve branch density (CNBD), corneal nerve fiber total branch density (CTBD), corneal nerve area (CNFA) and corneal nerve fiber fractal dimension (ACNFrD; \( P<0.001 \) for all)\textsuperscript{[38]}. Villani et al\textsuperscript{[36]} have reported similar results.

Corneal sensitivity Patients with DES showed lower corneal sensitivity when compared with control subjects, and is measured by both Belmonte noncontact gas esthesiometer and Cochet-Bonnet esthesiometer\textsuperscript{[37,39]}. The work of Achtsidis et al\textsuperscript{[40]} have revealed a significant reduction in the corneal sensitivity in the early GO, showing an association with the DES. Although the decreased corneal sensitivity in Villani et al\textsuperscript{[36]} study has no statistical difference between GO and NC, it showed correlation with propptosis (\( P<0.001 \)).

Corneal biomechanical properties Moghimi et al\textsuperscript{[41]} have used ocular response analyzer (ORA) to evaluate the variations of corneal biomechanical properties in GO. The study revealed that corneal hysteresis (CH) in GO patients is significantly lower than healthy subjects. Karabulut et al\textsuperscript{[42]} have come to similar conclusion.

 Conjunctiva

 In vivo confocal microscopy Wei et al\textsuperscript{[13]} have used in vivo confocal microscopy to observe the bulbar conjunctiva in GO patients as well as control subjects and revealed that GO patients suffered from more severe damage and inflammation in bulbar conjunctiva, especially in the superior area. They found that the superficial epithelial cell density in GO patients was significantly lower than NCs, with higher LC density and lower goblet cell density.

 Impression cytology Ismailova et al\textsuperscript{[20]} have selected 15 GO patients with positive vital staining to undergo impression cytology and incisional biopsy. They discovered goblet cell loss, significant epithelial dystrophy, and epithelial keratinization and leukocytic infiltration. Wei et al\textsuperscript{[15]} have also reported a significant decrease in goblet cells and increased LCs in GO patients. Squamous cell metaplasia was found in this study as well.

 Glands

 Meibomian gland Because of incomplete blinking caused by proptosis and eyelid retraction, GO patients are considered to face Meibomian gland dysfunction (MGD)\textsuperscript{[22]}. LipView is a technique that displays the structure, morphology and function of Meibomian gland (MG).

Park et al\textsuperscript{[43]} have used LipView to measure the lipid layer thickness, meibography and incomplete blinking rate of GO. They demonstrated that CAS showed a positive correlation with Meibo-score and the MGD might be a meaningful reason for the cause of DES. Park and Back\textsuperscript{[44]} have demonstrated that the incomplete blinking rate and MG loss in GO patients were significantly more severe than those with nonthyroidal dry eye.

 Lacrimal gland Due to physiological expression of THSR, lacrimal glands are considered to be the target of pathogenic antibodies in GO\textsuperscript{[45]}. Huh et al\textsuperscript{[46]} have described increased volume of lacrimal glands in Korean GO patients when compared to healthy subjects by measuring CT images (\( P<0.001 \)). In Huang et al\textsuperscript{[47]} study, the coronal and axial lacrimal gland areas were significantly larger in GO patients than NC. Also, the axial area of lacrimal glands showed positive correlation with the concentrations of IL-1β and IL-17A in tears, and coronal area with IL-6. Harris et al\textsuperscript{[48]} have pointed out that the enlargement of lacrimal glands showed low correlation with smoking, proptosis and inflammatory activity.

Subjective Changes

 Dry eye questionnaire The ocular surface disease index (OSDI) questionnaire is a global assessment tool for assessing the score of subjective feeling of dry eye from 0-100. This questionnaire consists of 12 questions and is filled by the patients, and is often used in GO patients to evaluate the patients’ ocular surface condition before or after using drugs\textsuperscript{[10,15,49]}. Meanwhile, OSDI score is higher in inflammatory diseases\textsuperscript{[30]}. GO patients often complain about their subjective feelings including asthenopia, foreign body sensation, photophobia, burning sensation and dryness of eyes. A total of 60 people (40 GO patients and 20 healthy volunteers) were enrolled in Wu et al\textsuperscript{[21]} study. GO patients were separated into 2 groups based on CAS. The study showed that both active and inactive GO patients had dry eyes and their OSDI (50.00, 27.08, separately) scores were significantly higher than those of healthy controls (0, \( P=0.00 \)). Other studies have also indicated similar phenomenon\textsuperscript{[15,17,22,36]}. In addition, other questionnaire might also be used in GO. Achtsidis et al\textsuperscript{[40]} have recorded subjective symptoms of dry eyes by International Dry Eye Working Group criteria using Schein’s questionnaire, and found that 67.8% active GO patients and 13.5% healthy controls (\( P<0.001 \)) suffer from DES. However, few studies have shown negative results. Wang et al\textsuperscript{[51]} have evaluated the ocular surface dryness by the Standard Patient Evaluation of Eye Dryness (SPEED) questionnaire. Only one patient with DES showed no statistical difference in this study, which is not only between the groups of GO patients and non-GO controls, but also between active and inactive GO. However, this study included only patients whose CAS was <4 and some participants in the control group
had DES and MG dysfunction, which could be the reason for the differences in conclusion.

**Quality of life** DES is the most common reason for eye discomfort in GO patients, influencing their quality of life. The GO-QOL questionnaire is designed to assess The quality of life (QOL) of GO patients through two main subscales: visual functioning and appearance, which has been confirmed and widely in use[32].

Lin et al[33] have conducted this questionnaire in 271 GO patients. They found that the score in visual functioning (58.39±25.17) and appearance (54.45±20.52) showed significant correlation with activity and disease severity.

In addition, GO patients might pay too much attention to their changed appearance and reduced social interactions, making their quality of life even inferior to those patients with diabetes or heart failure[36].

**Analysis for high occurrence of DES in GO** DES due to GO is a kind of DES, and involves features that of special endocrine disease. As mentioned above, DES occurs in 65%-85% GO patients, while there is no exact pathogenesis to explain how this occurs[36].

GO is an autoimmune disease that is mainly caused by antibodies targeting TSHR[9-10]. TSHR not only expresses in the thyroid but also in the orbit tissue including fibroblasts, lacrimal gland, etc[45]. Due to cross-reactivity antigens, autoimmune inflammatory responses occur in the orbit.

Damage to lacrimal glands causes swelling and influences its secretory function[47]. Wang et al[41] have indicated that active GO patients have more severe MGD and higher lipid layer thickness caused by periglandular inflammation of MG. Meanwhile, leukocytic infiltration, goblet cells loss and LCs increase also indicates inflammatory changes in the conjunctiva[20]. In addition, upregulation of inflammatory proteins and downregulation of protective proteins in tears might represent disease activity[32]. The changes of cytokines in tears of GO patients are also associated with disease activity and ocular surface damage. Increased levels of oxidative stress markers indicate imbalanced state of ocular surface. The changes in tear composition reflects biological modifications associated with GO[43] and might reveal their correlation with both autoimmune as well as inflammatory responses during the start and progression of GO. Therefore, the autoimmune responses brought by thyroid associated disease make DES special in GO. As known, GO involves the orbit and leads to a series of symptoms such as proptosis, eyelid swelling, eyelid retraction etc[12]. These changes leads to extra exposure of the ocular surface and incomplete blinking, causing increased osmolarity and damage to the cornea and conjunctiva[15-16].

Decreased corneal sensitivity and corneal epithelium injury might also occur. Furthermore, the eye movement disorder might increase the risk of corneal damage.

As mentioned above, the autoimmune responses that take place in GO patients result in extra ocular surface exposure, secretory imbalance of glands, changed composition of tears and unstable state of ocular surface, which are regarded as risk factors for the cause of DES. Therefore, DES has high prevalence in GO patients.

**CONCLUSION**

Therefore, the changes in each component of ocular surface might result in the occurrence of DES in GO patients (Table 1), featuring autoimmune inflammatory response and mechanically exposed ocular surface. There are no drugs targeting DES caused by

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Table 1 The ocular surface changes in Graves’ ophthalmopathy patients compared with normal controls
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GO and no standardized treatment guidelines. More studies should be conducted to figure out the key factors and how to prevent and cure this syndrome to improve the quality of life in GO patients.

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