• Review Article •

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 pathogenesisis 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 I 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*[15] 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 patients[17,24]. For example, Iskeleli *et al*'s[25] work mentioned that the tear film osmolarity that is measured by an auto-osmometer (OM-6030 AUTO STAT; Daiichi, Kyoto, Japan) in GO patients (340.38±18.74 mOsm) is significantly different from that of healthy subjects (290.80±13.58 mOsm/L, P=0.0001). The increased tear film osmolarity might be caused by proptosis and higher palpebral fissure. However, a recent study conducted by Kashkouli *et al*[26] have provided an inconsistent conclusion that even though 16.2% eyes of moderate-severe GO patients showed abnormal osmolarity, the mean osmolarity measured by TearLab device was 295.9 mOsm/L, which might be due to the differences in the detection methods.

Composition of tears For autoimmune and inflammatory reactions that take place in the orbit of GO patients, many studies have tested the amount of proteins and cytokines in the 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[23].

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*[32], 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*[33] have reported a significant increase of lysozyme C (LYZ), lacritin (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 sRI, and IL-13 was also reported.

Furthermore, Choi *et al*[34] have reported increased levels of oxidative stress markers, 8-hydroxy-2'-deoxyquanosine (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

index score and Schirmer test scores. According to a recent study conducted by Wu *et al*[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 fiber area (CNFA) and corneal nerve fiber fractal dimension (ACNFrD; $P<0.001$ for all)[38]. Villani *et al*[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[37,39]. The work of Achtsidis *et al*[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*'s[36] study has no statistical difference between GO and NC, it showed correlation with proptosis ($P<0.001$).

Corneal biomechanical properties Moghimi *et al*[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*[42] have come to similar conclusion.

Conjunctiva

***In vivo* confocal microscopy** Wei *et al*[15] 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*[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*[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)[22]. LipView is a technique that displays the structure, morphology and function of Meibomian gland (MG).

Park *et al*[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 Baek[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[45].

Huh *et al*[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*'s[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*[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[10,15,49]. Meanwhile, OSDI score is higher in inflammatory diseases[50].

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*'s[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[15,17,22,36].

In addition, other questionnaire might also be used in GO. Achtsidis *et al*[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*[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

Table 1 The ocular surface changes in Graves' ophthalmopathy patients compared with normal controls

Index	Graves ophthalmopathy patients compared with normal controls	Meaning
The tear breakup time	↓	Change of the tear quality
Schirmer's test	↓	Change of the tear quantity
The tear film osmolarity	↑	High evaporation rate
Proteins in tears	Inflammatory proteins ↑ Protective proteins ↓	The occurrence of inflammation
Cytokines in tears	Inflammatory cytokines ↑	Damaged balance of cytokines
Oxidative stress markers in tears	↑	Occurrence of the oxidative stress response
Corneal fluorescein staining score	↑	Damage to the cornea
Cornea *in vivo* confocal microscopy	Langerhans cells↑ The central corneal subbasal nerve plexus↓	Inflammation response and damage to corneal nerves
Corneal sensitivity	↓	Easier to get hurt
Corneal biomechanical properties	Corneal hysteresis↓	Change of corneal biomechanical properties
Conjunctiva *in vivo* confocal microscopy	Superficial epithelial cell density↓	Taking place of the inflammation and damage to the conjunctiva
Impression cytology	Goblet cells↓ Langerhans cells↑	Inflammation injury
Meibomian gland	Incomplete blinking rate↑ MG loss↑	Meibomian gland dysfunction
Volume of lacrimal glands	↑	Inflammation response

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[52].

Lin *et al*[53] 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[54].

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[16].

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*[51] 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[55] 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*[1-2]. 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

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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REFERENCES

1 Smith TJ, Hegedüs L. Graves' disease. *N Engl J Med* 2016;375(16):1552-1565.

2 Wang Y, Smith TJ. Current concepts in the molecular pathogenesis of thyroid-associated ophthalmopathy. *Invest Ophthalmol Vis Sci* 2014;55(3):1735-1748.

3 Smith TJ. Challenges in orphan drug development: identification of effective therapy for thyroid-associated ophthalmopathy. *Annu Rev Pharmacol Toxicol* 2019;59:129-148.

4 Mohyi M, Smith TJ. IGF1 receptor and thyroid-associated ophthalmopathy. *J Mol Endocrinol* 2018;61(1):T29-T43.

5 Smith TJ, Janssen JA. Building the case for insulin-like growth factor receptor-I involvement in thyroid-associated ophthalmopathy. *Front Endocrinol (Lausanne)* 2016;7:167.

6 Smith TJ, Janssen JAMJL. Insulin-like growth factor-I receptor and thyroid-associated ophthalmopathy. *Endocr Rev* 2019;40(1):236-267.

7 Fang SJ, Huang YZ, Liu XT, Zhong SS, Wang NJ, Zhao BB, Li YW, Sun J, Wang Y, Zhang S, Gu P, Zhou HF, Li B, Fan XQ. Interaction between CCR6+ Th17 cells and CD34+ fibrocytes promotes inflammation: implications in Graves' orbitopathy in Chinese population. *Invest Ophthalmol Vis Sci* 2018;59(6):2604-2614.

8 Fang SJ, Huang YZ, Wang SW, Zhang YD, Luo XR, Liu LY, Zhong SS, Liu XT, Li D, Liang R, Miranda P, Gu P, Zhou HF, Fan XQ, Li B. IL-17A exacerbates fibrosis by promoting the proinflammatory and profibrotic function of orbital fibroblasts in TAO. *J Clin Endocrinol Metab* 2016;101(8):2955-2965.

9 Bartalena L. Prevention of Graves' ophthalmopathy. *Best Pract Res Clin Endocrinol Metab* 2012;26(3):371-379.

10 Bahn RS. Graves' ophthalmopathy. *N Engl J Med* 2010;362(8):726-738.

11 Hiromatsu Y, Eguchi H, Tani J, Kasaoka M, Teshima Y. Graves' ophthalmopathy: epidemiology and natural history. *Intern Med* 2014;53(5):353-360.

12 Barrio-Barrio J, Sabater AL, Bonet-Farriol E, Velázquez-Villoria Á, Galofré JC. Graves' ophthalmopathy: VISA versus EUGOGO classification, assessment, and management. *J Ophthalmol* 2015;2015:249125.

13 Bartalena L, Baldeschi L, Dickinson AJ, *et al*. Consensus statement of the European group on Graves' orbitopathy (EUGOGO) on management of Graves' orbitopathy. *Thyroid* 2008;18(3):333-346.

14 Nelson JD, Craig JP, Akpek EK, *et al*. TFOS DEWS II introduction. *Ocul Surf* 2017;15(3):269-275.

15 Wei YH, Chen WL, Hu FR, Liao SL. *In vivo* confocal microscopy of bulbar conjunctiva in patients with Graves' ophthalmopathy. *Taiwan Yi Zhi* 2015;114(10):965-972.

16 Wiersinga WM, Bartalena L. Epidemiology and prevention of Graves' ophthalmopathy. *Thyroid* 2002;12(10):855-860.

17 Selter JH, Gire AI, Sikder S. The relationship between Graves' ophthalmopathy and dry eye syndrome. *Clin Ophthalmol* 2015;9:57-62.

18 Stapleton F, Alves M, Bunya VY, Jalbert I, Lekhanont K, Malet F, Na KS, Schaumberg D, Uchino M, Vehof J, Viso E, Vitale S, Jones L. TFOS DEWS II epidemiology report. *Ocul Surf* 2017;15(3):334-365.

19 Achtsidis V, Tentolouris N, Theodoropoulou S, Panagiotidis D, Vaikoussis E, Saldana M, Gouws P, Theodossiadis PG. Dry eye in Graves ophthalmopathy: correlation with corneal hypoesthesia. *Eur J Ophthalmol* 2013;23(4):473-479.

20 Ismailova DS, Fedorov AA, Grusha YO. Ocular surface changes in thyroid eye disease. *Orbit* 2013;32(2):87-90.

21 Wu LQ, Cheng JW, Cai JP, Le QH, Ma XY, Gao LD, Wei RL. Observation of corneal Langerhans cells by *in vivo* confocal microscopy in thyroid-associated ophthalmopathy. *Curr Eye Res* 2016;41(7):927-932.

22 Kim YS, Kwak AY, Lee SY, Yoon JS, Jang SY. Meibomian gland dysfunction in Graves' orbitopathy. *Can J Ophthalmol* 2015;50(4):278-282.

23 Huang DP, Xu N, Song YY, Wang PJ, Yang HS. Inflammatory cytokine profiles in the tears of thyroid-associated ophthalmopathy. *Graefes Arch Clin Exp Ophthalmol* 2012;250(4):619-625.

24 Gilbard JP, Farris RL. Ocular surface drying and tear film osmolarity in thyroid eye disease. *Acta Ophthalmol (Copenh)* 1983;61(1):108-116.

25 Iskeleli G, Karakoc Y, Abdula A. Tear film osmolarity in patients with thyroid ophthalmopathy. *Jpn J Ophthalmol* 2008;52(4):323-326.

26 Kashkouli MB, Alemzadeh SA, Aghaei H, Pakdel F, Abdolalizadeh P, Ghazizadeh M, Moradpasandi F. Subjective versus objective dry eye disease in patients with moderate-severe thyroid eye disease. *Ocul Surf* 2018;16(4):458-462.

27 Kishazi E, Dor M, Eperon S, Oberic A, Turck N, Hamedani M. Differential profiling of lacrimal cytokines in patients suffering from thyroid-associated orbitopathy. *Sci Rep* 2018;8(1):10792.

28 Ujhelyi B, Gogolak P, Erdei A, Nagy V, Balazs E, Rajnavolgyi E, Berta A, Nagy EV. Graves' orbitopathy results in profound changes in tear composition: a study of plasminogen activator inhibitor-1 and seven cytokines. *Thyroid* 2012;22(4):407-414.

29 Chen Q. The expression of interleukin-15 and interleukin-17 in tears and orbital tissues of Graves ophthalmopathy patients. *J Cell Biochem* 2019;120(4):6299-6303.

30 Cai KB, Wei RL. Interleukin-7 expression in tears and orbital tissues of patients with Graves' ophthalmopathy. *Endocrine* 2013;44(1):140-144.

31 Song RH, Wang B, Yao QM, Li Q, Jia X, Zhang JN. Proteomics screening of differentially expressed cytokines in tears of patients with Graves' ophthalmopathy. *Endocr Metab Immune Disord Drug Targets* 2020;20(1):87-95.

32 Matheis N, Grus FH, Breitenfeld M, Knych I, Funke S, Pitz S, Ponto KA, Pfeiffer N, Kahaly GJ. Proteomics differentiate between thyroid-associated orbitopathy and dry eye syndrome. *Invest Ophthalmol Vis Sci* 2015;56(4):2649-2656.

33 Aass C, Norheim I, Eriksen EF, Børnick EC, Thorsby PM, Pepaj M. Establishment of a tear protein biomarker panel differentiating between Graves' disease with or without orbitopathy. *PLoS One* 2017;12(4):e0175274.

34 Choi W, Li Y, Ji YS, Yoon KC. Oxidative stress markers in tears of patients with Graves' orbitopathy and their correlation with clinical activity score. *BMC Ophthalmol* 2018;18(1):303.

35 Li YW, Wei J, Shang LX, Zeng W. Clinical study of dry eye prevalence and tear film function and corneal status in patients with thyroid associated ophthalmopathy. *Guoji Yanke Zazhi (Int Eye Sci)* 2018;18(2):313-315.

36 Villani E, Viola F, Sala R, Salvi M, Mapelli C, Currò N, Vannucchi G, Beck-Peccoz P, Ratiglia R. Corneal involvement in Graves' orbitopathy: an *in vivo* confocal study. *Investig Ophthalmol Vis Sci* 2010;51(9):4574-4578.

37 Toker E, Asfuroğlu E. Corneal and conjunctival sensitivity in patients with dry eye: the effect of topical cyclosporine therapy. *Cornea* 2010;29(2):133-140.

38 Wu LQ, Mou P, Chen ZY, Cheng JW, Le QH, Cai JP, Wei RL. Altered corneal nerves in Chinese thyroid-associated ophthalmopathy patients observed by *in vivo* confocal microscopy. *Med Sci Monit* 2019;25:1024-1031.

39 Bourcier T, Acosta MC, Borderie V, Borrás F, Gallar J, Bury T, Laroche L, Belmonte C. Decreased corneal sensitivity in patients with dry eye. *Invest Ophthalmol Vis Sci* 2005;46(7):2341-2345.

40 Achtsidis V, Tentolouris N, Theodoropoulou S, Panagiotidis D, Vaikoussis E, Saldana M, Gouws P, Theodossiadis PG. Dry eye in Graves ophthalmopathy: correlation with corneal hypoesthesia. *Eur J Ophthalmol* 2013;23(4):473-479.

41 Moghimi S, Safizadeh M, Mazloumi M, Hosseini H, Vahedian Z, Rajabi MT. Evaluation of corneal biomechanical properties in patients with thyroid eye disease using ocular response analyzer. *J Glaucoma* 2016;25(3):269-273.

42 Karabulut GO, Kaynak P, Altan C, Ozturker C, Aksoy EF, Demirok A, Yılmaz OF. Corneal biomechanical properties in thyroid eye disease. *Kaohsiung J Med Sci* 2014;30(6):299-304.

43 Park J, Kim J, Lee H, Park M, Baek S. Functional and structural evaluation of the meibomian gland using a LipiView interferometer in thyroid eye disease. *Can J Ophthalmol* 2018;53(4):373-379.

44 Park J, Baek S. Dry eye syndrome in thyroid eye disease patients: The role of increased incomplete blinking and Meibomian gland loss. *Acta Ophthalmol* 2019;97(5):e800-e806.

45 Eckstein AK, Finkenrath A, Heiligenhaus A, Renzing-Köhler K, Esser J, Krüger C, Quadbeck B, Steuhl KP, Gieseler RK. Dry eye syndrome in thyroid-associated ophthalmopathy: lacrimal expression of TSH receptor suggests involvement of TSHR-specific autoantibodies. *Acta Ophthalmol Scand* 2004;82(3 Pt 1):291-297.

46 Huh HD, Kim JH, Kim SJ, Yoo JM, Seo SW. The change of lacrimal gland volume in Korean patients with thyroid-associated ophthalmopathy. *Korean J Ophthalmol* 2016;30(5):319-325.

47 Huang DP, Luo Q, Yang HS, Mao YX. Changes of lacrimal gland and tear inflammatory cytokines in thyroid-associated ophthalmopathy. *Invest Ophthalmol Vis Sci* 2014;55(8):4935-4943.

48 Harris MA, Realini T, Hogg JP, Sivak-Callcott JA. CT dimensions of the lacrimal gland in Graves orbitopathy. *Ophthalmic Plast Reconstr Surg* 2012;28(1):69-72.

49 Gürdal C, Genç I, Saraç O, Gönül I, Takmaz T, Can I. Topical cyclosporine in thyroid orbitopathy-related dry eye: clinical findings, conjunctival epithelial apoptosis, and MMP-9 expression. *Curr Eye Res* 2010;35(9):771-777.

50 Garcia DM, Reis de Oliveira F, Módulo CM, Faustino J, Barbosa AP, Alves M, Rocha EM. Is Sjögren's syndrome dry eye similar to dry eye caused by other etiologies? Discriminating different diseases by dry eye tests. *PLoS One* 2018;13(12):e0208420.

51 Wang CY, Ho RW, Fang PC, Yu HJ, Chien CC, Hsiao CC, Kuo MT. The function and morphology of Meibomian glands in patients with thyroid eye disease: a preliminary study. *BMC Ophthalmol* 2018;18(1):90.

52 Wiersinga WM. Quality of life in Graves' ophthalmopathy. *Best Pract Res Clin Endocrinol Metab* 2012;26(3):359-370.

53 Lin IC, Lee CC, Liao SL. Assessing quality of life in Taiwanese patients with Graves' ophthalmopathy. *J Formos Med Assoc* 2015;114(11):1047-1054.

54 Bruscolini A, Sacchetti M, la Cava M, Nebbioso M, Iannitelli A, Quartini A, Lambiase A, Ralli M, de Virgilio A, Greco A. Quality of life and neuropsychiatric disorders in patients with Graves' Orbitopathy: current concepts. *Autoimmun Rev* 2018;17(7):639-643.

55 Kishazi E, Dor M, Eperon S, Oberic A, Hamedani M, Turck N. Thyroid-associated orbitopathy and tears: a proteomics study. *J Proteomics* 2018;170:110-116.