Assessment of Meibomian Gland (MD) Impairment Among Seasonal Allergic Conjunctivitis (SAC) Patients

Background: Allergic conjunctivitis, one of the frequently occurring ocular surface diseases, can cause mucus discharge, itchy sensation, conjunctival hyperemia, and papillary formation. Seasonal allergic conjunctivitis (SAC) is associated with xerophthalmia and instability of tear film. Meibomian gland (MG) can secrete lipids to avoid xerophthalmia. However, there have been few reports on MG morphological alterations of SAC patients. This study aimed to examine the morphological alterations of MG among SAC patients.

Material/Methods: Our study included 89 eyes from 89 patients with SAC and 112 eyes of healthy volunteers. The symptoms were assessed by ocular surface disease index (OSDI) questionnaire. Then, the tests shown below were carried out, including tear evaporation rate from the ocular surface (TEROS), slit-lamp examination, break-up time (BUT) of tear film, Schirmer test I, vital staining, meibography, and meibum expression grading. MG was examined with laser scanning confocal microscopy (LSCM).

Results: Relative to the control group, the OSDI was significantly higher in the SAC group. TEROS values, BUT, vital staining, MG expression, MG distortion rates, and MG dropout grades were significantly worse in the SAC group compared with the control group. As suggested by LSCM, SAC patients had markedly worse averages of parameters compared with controls.

Conclusions: The patients with SAC have more significant morphological and cytological changes in the MG. The Keratograph 5M system and LSCM are effective methods for evaluating MG status and ocular surface diseases.

Keywords: Conjunctivitis, Allergic • Meibomian Glands • Microscopy, Confocal

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Background

Allergic conjunctivitis (AC) is a common ocular surface disease [1]. AC encompasses a group of subtypes, such as perennial or seasonal allergic conjunctivitis (PAC or SAC), and atopic or vernal keratoconjunctivitis (AKC or VKC). It is suggested that AC is related to xerophthalmia and instability of tear film [2]. Typically, SAC is the most common subtype of AC, accounting for 90% of all cases. As suggested by Ayaki et al [3], SAC was related to worse tear film instability and an aberrant tear film lipid layer. The number of SAC patients has been increasing yearly [4]. Additionally, SAC patients tend to develop xerophthalmia [5].

Xerophthalmia is the instability of tear film, and can injure the ocular surface [6]. Tear film is composed of water, mucins, and lipids. The meibomian gland (MG) can secrete lipids, which are the major components in the tear film lipid layer, which exert an important role in surface tension, stabilizing tear film, and preventing evaporation [7,8]. The abnormal or disordered MGs can result in instability of tear film or fast tear evaporation, thereby elevating the tear osmolarity and finally leading to xerophthalmia [9].

Previous studies have reported the ocular surface disorders in PAC, AKC, and VKC patients. However, there are few studies on xerophthalmia in SAC patients. In this study, the xerophthalmia in patients with SAC was analyzed, as well as the associations with alterations of ocular surface, so as to further examine the pathogenic mechanism of xerophthalmia in SAC and provide guidance for clinical diagnosis and treatment.

Material and Methods

Study subjects

We evaluated 89 eyes of 89 patients with recurrent SAC for more than 3 years (40 men and 49 women; average age, 30.5±15.5 years). SAC was clinically diagnosed on the base of patients’ feeling an itchy sensation and papillary formation in the conjunctiva. As a control group, 112 eyes from 112 healthy volunteers (55 men and 57 women; average age, 28.5±12.5 years) were included. For all participants, their right eyes were examined. Exclusion criteria were: patients with histories of blepharitis, trauma, eye surgery, ocular inflammation, persistent use of eye drops, and Sjogren syndrome, as well as ocular or systemic disorder potentially interfering with tear film generation or function. Each participant provided written informed consent prior to participation in this study, and the Ethics Committee of Shandong Provincial Weifang Eye Hospital approved our study protocol.

Symptom Assessment

Ocular surface symptoms, together with the xerophthalmia degree, were evaluated using the ocular surface disease index (OSDI). The questionnaire total score ranges from 0 (no symptoms) to 100 (severe symptoms), and a higher score indicates more severe xerophthalmia symptoms [10]. Generally, xerophthalmia was determined when the OSDI score was ≥13 [11].

Tear Evaporation Rate Measurement

A quartz crystal humidity sensor (Kao Analytical Research Center, Tochigi, Japan) was used to assess the tear evaporation rate from the ocular surface (TEROS). The temperature and humidity throughout our experimental period were kept at 20-25°C and 30-50%, respectively [12].

Stability of Tear Film and Ocular Surface Staining Examination

Fluorescein (2 μl) was instilled to measure the break-up time (BUT) for the tear film. To ensure sufficient dye mixing, subjects were instructed to blink their eyes repeatedly. The duration from the final blink to the initial rupture occurrence was determined 3 times [13]. Typically, BUT <5 s indicates abnormality [14]. To assess fluorescein staining, we classified the cornea as 3 zones: upper, middle, and lower. There was 1 superficial punctate keratopathy (SPK) score (range, 0-3 points) for every zone. The total score varied from 0 to 9 points, and a score >3 indicated abnormality [15].

Tear Quantity Assessment

The generation of tear film was evaluated by Schirmer test I with no surface anesthetics. During the test period, the test strips (Showa Yakuhin Kako Co., Ltd., Tokyo, Japan) were put into the lateral canthus for 5 min. Then, all data were documented in millimeters of wetting [16]. Typically, a value <5 mm indicates abnormality.

Keratography 5M Evaluation

The MG distortion and MG dropout were detected with the Keratograph 5M system (Oculus GmbH, Wetzlar, Germany). Upper and lower eyelids were subjected to eversion by non-contact meibography, and MG was also assessed. When a greater than 45-degree distortion was detected in 1 or more MG ducts within the upper and lower eyelids, MG distortion was confirmed. The score of MG distortion was rated as 0-2: 0 indicated no distortion; 1 indicated distortion of 1-4 MGs; while 2 indicated distortion of over 5 MGs [17]. For every eyelid, partial or total MG loss was assessed by grades: 0 indicated no MG loss; 1 indicated <1/3 total MG area loss; 2 indicated <2/3
total MG area loss; while 3 indicated >2/3 total MG area loss. Thereafter, the meibography score of the upper eyelid was added to that of the lower eyelid, yielding the total score of every eye (range, 0-6), and the score >1 was considered abnormal [18].

**MG Expression Assessment**

Expression of MG was evaluated semi-quantitatively based on Bron’s grading [19]. We also assessed the glandular orifice (including narrowing, capping, obliteration level, cuffing loss, opaque/scared, pouting) and the secretion expressed (clear, cloudy, or toothpaste) [20].

**Laser Scanning Confocal Microscopy (LSCM)**

LSCM was performed using the Heidelberg Retina Tomograph III-Rostock Cornea Module (Heidelberg Engineering GmbH, Dossenheim, Germany). During eyelid eversion, central Tomo-Cap was plated on the palpebral conjunctiva; later, the flat lens was moved to examine MGs [21]. The LSCM examination was performed by the same operator for all subjects. Three randomized, non-overlapping, digital images with high quality were obtained for each temporal, middle, or nasal lower eyelid (9 images for each eyelid in total), and they were then used to calculate CM parameters.

To evaluate those morphological alterations of MGs, the periglandular inflammatory cell density (ICD), meibomian gland acinar unit atrophy and density (MGAUA and MGAUD), and meibomian gland acinar longest and shortest diameter (MGALD and MGASD) were determined [22]. Among them, ICD and MGAUD were detected using specific software. MGAUA was evaluated by plotting 1 line surrounding the apparent acinar unit inner lumen. MGAUD was labeled with hands for every 400×400 μm frame computed by Cell Count automatically, whereas MGALD and MGASD were measured using Image J software (http://rsb.info.nih.gov/ij/).

**Statistical Methods**

Statistical analysis was performed using SPSS 23.0 (SPSS Inc., Chicago, IL, USA). The difference in sex ratio was compared between SAC patients and normal controls. Differences in various examinations (including OSDI, TEROS, punctate keratopathy SPK score, BUT, meibography score, Shirmer test I, ICD values, MGAUA, MGAUD, MGALD, and MGASD) between the patients and normal controls were assessed by Mann-Whitney U test. A difference of \( P < 0.05 \) indicated statistical significance.

### Results

**Evaluation of Xerophthalmia**

Xerophthalmia was assessed by the OSDI. Our results showed that 92.13% of the SAC patients had xerophthalmia, in comparison with the 29% of the control subjects. Moreover, the OSDI of SAC patients was clearly higher than in normal controls (\( P < 0.05 \)) (Table 1).

| Parameters          | SAC (n=89)       | Controls (n=112) | P value |
|---------------------|------------------|------------------|---------|
| OSDI (points)       | 21.03±6.21       | 11.22±5.72       | <0.05   |
| TEROS (10^-7 g/cm^2/s) | 10.4±4.0        | 3.5±1.1          | <0.05   |
| BUT (s)             | 5.2±2.4          | 8.4±2.3          | <0.05   |
| Schirmer I test (mm)| 10.8±6.8         | 13.5±6.2         | >0.05   |
| SPK score (points)  | 4.4±1.9          | 0.3±0.6          | <0.05   |

**Tear evaporation Rate Measurement**

We determined and compared the tear evaporation rate, showing that, relative to normal controls, SAC patients had higher TEROS (\( P < 0.05 \)) (Table 1).

**Stability of Tear Film and Ocular Surface Staining Examination**

As shown by tear stability and ocular surface staining tests, SAC patients had significantly lower mean BUT and SPK scores relative to the control group (\( P < 0.05 \)) (Table 1).

**Tear Quantity Evaluation**

Tear quantity assessment showed there was not a significant difference between SAC and control groups in the averages obtained from the Schirmer I test (\( P > 0.05 \)) (Table 1).
Table 2. Disease grading of the study subjects.

| Grade          | SAC       | Controls | P value |
|----------------|-----------|----------|---------|
| MG distortion  | 0.32±0.5  | 0.11±0.33| <0.05   |
| MG dropout     | 1.8±0.5   | 0.3±0.6  | <0.05   |
| MG expression  | 1.9±0.5   | 0.2±0.3  | <0.05   |
| MG orifices    | 2.8±0.9   | 0.2±0.4  | <0.05   |

MG distortion, dropout, and expression grades were significantly worse in the SAC group than the control group. Eyelid evaluation in the SAC group was significantly worse than in the control group. The Mann-Whitney U test was performed for pairwise comparison.

Discussion

Lipids secreted by the MGs are the most important components in tear film, and they can prevent rapid evaporation of tear film, facilitate lubrication to reduce friction, and provide a smooth optical surface. Lack of lipids can lead to xerophthalmia [23]. It has been previously shown that SAC is associated with xerophthalmia and instability of tear film, yet little research has reported the MG morphological alterations of SAC patients. Thanks to the development of Keratography 5M and LSCM, alterations of ocular surface structure and function can be investigated in a non-invasive way. In the present study, these 2 methods were used to evaluate the tear function and MG alteration in SAC patients and normal control subjects. Although there are many similar studies, most are on changes of the meibomian gland related to perennial allergic conjunctivitis or giant papillary conjunctivitis, and relatively few studies are on changes of the meibomian gland caused by SAC. Thus, we further studied this disease.

In this study, according to OSDI analysis, 92.13% of SAC patients had xerophthalmia, compared to 29% of normal controls. The tear function and MG alteration in SAC patients and control subjects were also investigated. SAC patients had markedly decreased BUT and poor vital staining relative to normal controls. Compared with normal controls, SAC patients did not have remarkably smaller tear volume. It has been previously shown that the disturbance of tear quality and tear quantity in SAC patients are associated with a decreased goblet cell population in the conjunctiva [24]. Moreover, the disturbance of mucin secreted by goblet cells can lead to decreased tear film stability [25]. Our results showed that 60.67% of the SAC patients had MG dropout, compared to 26.79% among the normal controls. Additionally, the mean MG distortion scores of SAC patients were dramatically higher than in normal controls. Moreover, MG expression grading in the SAC patients was significantly worse than the normal control subjects. These results suggest that the symptom of xerophthalmia among SAC patients might be due to the changed tear film lipid layer, and this is possibly related to MG distortion, MG dropout, and changed MG orifices.
Several LSCM parameters are of significance in assessing histopathological changes in the MG, including MGAUA, MGAUD, MGALD, and MGASD. Moreover, the periglandular ICD could help to differentiate the inflammatory obstructive MG dysfunction from the non-inflammatory type. The above-mentioned parameters are sensitive and specific in diagnosing MG impairment, which are also strongly associated with ocular surface status [26].

In this study, the LSCM examination revealed that a large proportion of the SAC patients exhibited MG alterations, like acinar unit enlargement, non-regular shape with acinus, and elevated ICD, but had reduced acinar unit density. Therefore, it is hypothesized that MG ductal obstruction might exist in SAC patients. MG ductal obstruction can result in meibum accumulation, thus inducing cystic dilatation as well as the aberrant acinus morphology, and further inducing decreased acinar density. In addition, it has been suggested in a prior study

Figure 1. LSCM images of MG orifices and morphologic changes in SAC patient compared with normal control subject. (PPT, WPS office 2016, Kingsoft Software Company). (A) The MG orifices showed obvious obliteration and narrowing in an SAC patient. (B) Representative image of MG orifices in a normal control. (C) Representative LSCM image for MG in an SAC patient. MG acinar unit diameters were enlarged. (D) LSCM image of a normal subject displaying a number of compact MG acinar units.
that the tears in SAC patients contained inflammatory cytokines, such as IL-4, IL-5, or TNF-α [27]. They exert vital roles in maintaining the conjunctival inflammation response, indicating that the inflammatory cytokines may exert effects on pathological changes [27]. These findings indicate the role of MGs changes in the discomfort or instability of tear film among SAC patients.

The findings in this study are consistent with previous results that significant changes were detected in MGs of SAC patients [28]. However, the definite mechanism underlying MG changes in SAC patients remains unknown, and some hypotheses have been proposed, like MG infiltration by lymphocytes and MG ductal epithelial hyperkeratinization, and, more recently, the inflammatory damages to the cornea and conjunctiva [29]. Arita et al [7,8] reported the MG duct alterations among PAC and AC patients were related to wearing contact lens. Under both of these conditions, there would be inflammation in the conjunctiva, which can damage the corneal epithelium and affect corneal nerves. In SAC patients, the MG structure or functions on the ocular surface may be changed as a result of the direct inflammatory damages to the conjunctiva or cornea resulting from the dermatitis-induced increased inflammatory state, or because of the indirect effect from the elevated inflammatory cytokine and cell production. In addition, patients with SAC tend to rub their eyes frequently, which might induce MG duct distortion. We believe that the MG dysfunction would deteriorate the quantity and quality of the meibum, and this possibly aggravates the instability of tear film, thus causing worse xerophthalmia. The changed MGs can occur concurrently with SAC inflammation, such as the surface and tear alterations, but not in a sequential manner. In this study, our results showed that MG secretions were expressed at very low levels in SAC patients compared with normal control subjects, which was shown by the clearly increased expression grade. This might be related to the significant glandular distortion and loss in the SAC group.

Our study has some limitations. First, no significant correlation was noted between the subject age and MG loss, probably due to the fact that, in contrast to previous studies, the cohort in this study consisted of patients with symptomatic
ocular surface disease alone. Second, this study did not evaluate the geographical origin of those enrolled patients, which might make comparison with other studies difficult. Third, the MG alterations related to SAC are complex, and many factors are involved, like immune system damage and frequently rubbing the eyes.

Conclusions

In conclusion, changes of MG morphology were observed in SAC patients by use of Keratography 5M and LSCM. Our results provide new evidence of the MG pathological changes in SAC. The Keratography 5M and LSCM used herein were effective and non-invasive methods for evaluation of structural and functional changes of MGs in SAC patients. The SAC patients displayed obvious MG morphological changes and impairment relative to normal controls.

Declaration of Figures’ Authenticity

All figures submitted have been created by the authors, who confirm that the images are original with no duplication and have not been previously published in whole or in part.
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