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Comparison of tear film tests, ocular staining, impression cytology for three conditions: dry eye, anterior blepharitis, seasonal allergic conjunctivitis

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干眼症和前睑缘炎及季节性过敏性结膜炎中三种检测方法结果的比较

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摘要

目的:比较三组临床患者和对照组患者的泪液功能测试,眼表染色以及结膜印象细胞学检测的结果。

方法:这是一个单中心,前瞻性,双盲,随机对照试验。研究包括泪液分泌分数小于10mm和泪膜破裂时间(TBUT)小于10s的20例干眼症患者,有干燥和结痂睫毛的20例前睑缘炎患者,上眼睑睑板结膜乳头肥大的20例季节性过敏性结膜炎患者和20例对照组患者。比较泪液分泌的分数,泪膜破裂时间,眼表染色(牛津分级方案),杯状细胞密度(尼尔森分级方案评估所有患者的印象细胞学和化生分数)。

结果:三组患者和对照组患者在泪液功能测试结果,眼表染色评分,杯状细胞密度和化生分数上有显著差异($P<0.001$)。

结论:结果证明这三种疾病的炎症反应造成严重眼表损害,并在局部产生炎症。这种损伤产生非常严重的影响,尤其是对结膜杯状细胞密度和化生。杯状细胞损伤程度与临床研究结果有关联。

关键词:前睑缘炎;结膜印象细胞学;干眼症;眼球表面染色;季节性过敏性结膜炎

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Abstract

• AIM: To compare three clinically similar patient groups and a control group in terms of tear function tests, ocular surface staining and conjunctival impression cytology.

• METHODS: This was a single-centre, prospective, double-blind, randomised and controlled trial. The study includes 20 dry eye patients with Schirmer 1 scores less than 10mm and a tear film break-up time (TBUT) less than 10s, 20 anterior blepharitis patients with drying and crusting of the eyelashes, 20 seasonal allergic conjunctivitis patients with papillary hypertrophy of the upper eyelid tarsal conjunctiva and 20 control group patients. The Schirmer scores, TBUT scores, ocular surface staining (as graded by the Oxford scheme scale), goblet cell density (as observed using impression cytology and metaplasia scores for all patients evaluated by the Nelson grading scheme) were compared.

• RESULTS: Significant differences were identified between these patient groups and the control group in terms of tear functions tests, ocular surface-staining scores, goblet cell density and metaplasia scores ($P<0.001$).

• CONCLUSION: Inflammatory response against these three diseases was demonstrated to cause damage in parallel to the severity of the local inflammation they generate on the ocular surface. We confirmed that this damage has very serious effects, especially on conjunctival goblet cell density and metaplasia. We believe that the degree of this loss in goblet cells is correlated with clinical findings.

• KEYWORDS: anterior blepharitis; conjunctival impression cytology; dry eye; ocular surface staining; seasonal allergic conjunctivitis
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INTRODUCTION

Dry eye is a multifactorial disorder of the tear film and ocular surface that results in eye discomfort and ocular surface damage[1,2]. The International Dry Eye WorkShop defined it as a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to

the ocular surface. It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface[3]. Meibomian gland dysfunction is the leading cause of dry eye disease[2-4]. The diagnosis of dry eye basically depends on the history of the patient and the evaluation of tear function tests[1]. Blepharitis is an inflammatory disease of the eyelash roots and eyelids[5]. Based on anatomic involvement, this disease is categorised as anterior or posterior blepharitis. Abnormal bacterial colonisation, seborrheic dermatitis, and acne rosacea are frequently observed in the aetiology of blepharitis[5]. These bacteria directly or indirectly cause inflammation, which decreases the efficiency of the Meibomian glands and, as a result, disturbs the composition of the lipid layer of the tear film and causes a decrease in its production[5]. The diagnosis of blepharitis basically depends on the history of the patient and the examination of eyelash bottoms and eyelid margin[5]. Seasonal allergic conjunctivitis is an IgE - dependent mast cell disease and recurrent hypersensitivity reaction against specific airborne allergens[6-8]. Patients with seasonal allergic conjunctivitis present with itchiness, redness, serous effusion, and complaints of stinging or burning in both eyes in the spring or fall. The diagnosis of seasonal allergic conjunctivitis basically depends on the history of the patient and the examination. The presence of papillary hypertrophy in the conjunctiva strengthens the diagnosis[6].

SUBJECTS AND METHODS

Our study was performed at Pamukkale University, department of ophthalmology, between March 2009 and May 2009 and included 160 eyes: 20 dry eyes, 20 eyes with anterior blepharitis, 20 eyes with seasonal allergic conjunctivitis and 20 control eyes. Regarding the dry eye diagnostic criteria in prospectively evaluated cases, Schirmer test values <10mm and tear film break-up time (TBUT) values <10s (measured with timer) were used. Desiccation and crusting of the eyelashes and the presence of keratoconjunctivitis were accepted as the diagnostic criteria for anterior blepharitis. The seasonal allergic conjunctivitis diagnostic criteria included papillary hypertrophy of the upper eyelid tarsal conjunctiva and complaints of itching and burning. Patients without any of the aforementioned findings were included in the normal, healthy control group.

Patients with other known systemic diseases who were receiving drug treatment for systemic or ophthalmic diseases, had previously undergone ocular surgery, or had lid/lash deformities were excluded from the study. Before commencing the study, approval was obtained from the Medical Ethics Committee of our Hospital. The study was conducted in accordance with the tenets of the Declaration of Helsinki.

After each patient had read the informed consent form, written consent was obtained. All patients who participated in the study underwent a complete ophthalmic examination including visual acuity, intraocular pressure measurement, anterior segment examination, and fundus examination by the same physician (MA).

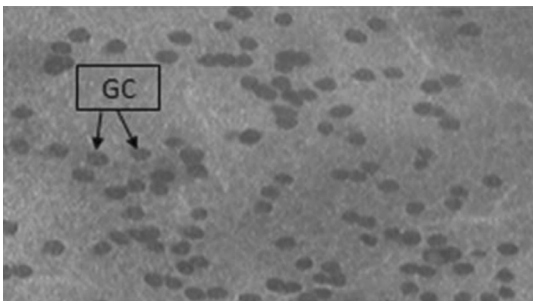

Figure 1 GC: Goblet cells, periodic acid Schiff (PAS) and hemalun stain.

Tear film break-up time Fluorescein-soaked strips (Bio Glo Sterile Strips, Rose Stone Enterprises, CA, USA) were wet with a standardised drop volume of non-preserved saline solution and were applied to the lower fornix. Following diffusion of the fluorescein, the tear film was examined using a blue cobalt filter. The time from the last blink to the appearance of the first dry spot was measured with timer. The measurement was repeated three times, and the mean value was calculated. A TBUT of less than 10s suggests an unstable tear film[1].

Cornealstaining with fluorescein Cornea was evaluated using a blue cobalt filter just after the TBUT measurement (one minute later the insertion of the stain). Corneal staining was evaluated using the six stages of the Oxford scheme[9].

Schirmer test A standard Schirmer filter paper strip (Tear Flo Sterile Strips, Rose Stone Enterprises, CA, USA) was inserted into the inferior lid margin 2-3mm from the lateral canthus without using a topical anaesthetic agent. After 5min, the moist part of the strip was measured starting from the edge of the eyelid. Less than 10mm of wetting after 5min indicates a diagnosis of tear-deficiency[1,10].

Conjunctivalstaining with Lissamine green Lissamine green-soaked strips (Lissamine green sterile strips, Rose Stone Enterprises, CA, USA) were wet with a standardised drop volume of non-preserved saline solution and were applied to the upper bulbar conjunctiva. Patients were asked to blink 3-4 times to allow the Lissamine to spread. An assessment was then conducted on the temporal conjunctival quadrant five minutes later the insertion of the stain using the yellow light on a slit-lamp microscope. Conjunctival staining was evaluated using the six stages of the Oxford scheme[9].

Conjunctival Impression Cytology Impression cytology samples were obtained with 0.022 and 0.025 micrometer pore-size filter paper (Sartorius AG, Göttingen, Germany), which were pressed to the superior temporal interpalpebral conjunctiva 2mm away from the limbus after applying topical anaesthesia (0.5% proparacaine hydrochloride, Alcon, Ft. Worth, TX, USA) using the method described by Egbert[11]. Samples were kept in 70% ethanol, 37% formaldehyde and a glacial acetic acid combination at 4° and then stained with periodic acid Schiff (PAS) and hemalun. The preparations were examined under the light microscope following the staining procedure[11-13]. Images of conjunctival goblet cells under a light microscope are given in Figure 1. Five randomly

Table 1 Demographics of the patients

Parameters	Dry eye (n=20)	Anterior blepharitis (n=20)	Seasonal allergic conjunctivitis (n=20)	Control (n=20)	P
F/M	13/7	12/8	15/5	17/3	0.05
Age	31.8±7.08	33.35±5.48	28.8±4.03	31.85±5.44	0.05

Table 2 Comparison of the Schirmer-I, TBUT, corneal fluorescein and conjunctival lissamine green staining ($\bar{x}\pm s$, range)

Parameters	Dry eye	Anterior blepharitis	Seasonal allergic conjunctivitis	Control	P (ANOVA)
Schirmer I (mm-5min)	4.15±1.81 (1-7)	8.80±2.91 (3-15)	[1]12.85±6.12 (4-25)	[1]15±3.06 (10-20)	<0.001
TBUT (s)	4.05±1.70 (1-7)	[2]6.90±2.53 (3-11)	[2]7.50±2.91 (3-11)	11.05±2.72 (6-16)	<0.001
Fluorescein staining (Oxford Scheme)	2.55±0.60 (2-4)	1.55±0.60 (1-3)	[3]0.70±0.73 (0-3)	[3]0.60±0.50 (0-1)	<0.001
Lissamine staining (Oxford Scheme)	2.55±1.09 (1-5)	[4]1.35±0.74 (0-3)	[4,5]0.80±0.95 (0-4)	[5]0.30±0.47 (0-1)	<0.001

[1-5]A statistically significant difference was not found between the groups (P>0.05 one-way ANOVA with Bonferroni correction); A statistically significant difference was found between the other groups (P<0.05 one-way ANOVA with Bonferroni correction).

Table 3 Comparison of goblet cell density and goblet cell metaplasia grading according to Nelson's classification ($\bar{x}\pm s$, range)

Parameters	Dry eye	Anterior blepharitis	Seasonal allergic conjunctivitis	Control	P(ANOVA)
Goblet cell density (cells-0.25mm^2)	61.75±21.31 (32-112)	[1]82.70±21.37 (36-110)	[1]89.6±16.83 (46-112)	170.60±24.72 (122-204)	<0.001
Goblet cell metaplasia grading	2.05±0.75 (1-3)	1.50±0.60 (1-3)	0.95±0.60 (0-2)	0.10±0.30 (0-1)	<0.001

[1]A statistically significant difference was not found between the groups (P>0.05 one-way ANOVA with Bonferroni correction); A statistically significant difference was found between the other groups (P<0.05 one-way ANOVA with Bonferroni correction).

selected areas of each sample were photographed. The number of goblet cells per 500×500 micrometer area was counted in five separate areas, and the outcomes were averaged to obtain a sample score[14]. The preparations were also staged for conjunctival epithelial squamous metaplasia using the method described by Nelson *et al*[15]. Impression cytology specimens were evaluated and graded by the same investigator (ACT) blinded to whether specimens were from a patient or a control.

Statistical Analysis The data were analysed using SPSS 10.0 software. One-way variance analysis (ANOVA) was instituted for the Schirmer, TBUT, corneal and conjunctival staining, goblet cell density (GCD) and goblet cell metaplasia staging data, Bonferroni correction was made after the ANOVA. The Chi-squared test was used for the demographic findings. The right and left eyes were first analysed separately. Because the groups were similar, only right eyes were used for our trial.

RESULTS

In our study, 20 dry eye patients, 20 anterior blepharitis patients, 20 seasonal allergic conjunctivitis patients and 20 control group patients were included. No statistically significant difference was observed between these three patient groups and the control group in terms of average age or gender (P>0.05) (Table 1).

A statistically significant difference was found between patient groups and the control group in terms of tear function tests, corneal staining with fluorescein and conjunctival staining with Lissamine green tests (P<0.001) (Table 2). A statistically significant difference was not found between seasonal allergic conjunctivitis and the control groups in terms of Schirmer I test (P>0.05 one-way ANOVA with Bonferroni correction). A statistically significant difference was not found between seasonal allergic conjunctivitis and the anterior blepharitis groups in terms of TBUT test (P>0.05 one-way ANOVA with Bonferroni correction). A statistically significant difference was not found between seasonal allergic conjunctivitis and the control groups in terms of Fluorescein staining (P>0.05 one-way ANOVA with Bonferroni correction). A statistically significant difference was not found between seasonal allergic conjunctivitis and the control groups, seasonal allergic conjunctivitis and anterior blepharitis in terms of Lissamine staining (P>0.05 one-way ANOVA with Bonferroni correction). A statistically significant difference was established between patient groups and the control group in terms of conjunctival impressions ofcytology goblet cell density and Nelson grading levels (P<0.001) (Table 3). A statistically significant difference was not found between seasonal allergic conjunctivitis and the anterior blepharitis groups in terms of GCD (P>0.05 one-way ANOVA with Bonferroni correction).

DISCUSSION

The ocular surface is affected to varying degrees in dry eye, anterior blepharitis, and seasonal allergic conjunctivitis[5,15-18].

In studies conducted by Perry *et al*[19] and Rubin and Rao[20] on chronic blepharitis, tear break-up time secondary to inflammation, and Schirmer test, the reported values were low. In our study, TBUT values and Schirmer test values of anterior blepharitis patients were also low. We believe that such low levels of tear function, GCD and high level of the goblet cell squamous metaplasia score could shed light on the secondary dry eye symptoms experienced by anterior blepharitis patients.

Studies conducted by Dogru[16], Bacon[17], and Toda *et al*[18] reported that patients developed a condition similar to dry eye due to goblet cell loss secondary to inflammation. Furthermore, metaplasia occurred in patients with allergic conjunctivitis, and the ocular surface was affected secondary to this allergic reaction. Although these authors established low TBUT values in allergic conjunctivitis, they reported Schirmer test values as near normal values due to the presence of irritation. Here, we found low TBUT values and near normal Schirmer test values compared to controls in our seasonal allergic conjunctivitis patients. We believe that such low TBUT levels and GCD in particular, could explain the mucin deficiency secondary to inflammation and the resulting dry eye found in allergic conjunctivitis patients.

The composition of the ocular surface has been reported to be affected to varying degrees in dry eye, anterior blepharitis, and allergic conjunctivitis[15-18]. In our study, the ocular surface staining with fluorescein and Lissamine green was highest in the dry eye group compared to the other groups. Fluorescein staining was not significant differ from in seasonal allergic conjunctivitis compared to control group, Lissamine staining was not significant differ from in seasonal allergic conjunctivitis compared to control and anterior blepharitis groups. We think that, ocular surface staining may reflect severity of the ocular surface diseases.

Conjunctival GCD and the morphological characteristics of conjunctival goblet cells are established by the examination of conjunctival impression cytology. By evaluating the morphological characteristics of conjunctival goblet cells, information is obtained on the maturation of the conjunctival epithelium, and if present, inflammation of the ocular surface[21]. Because the GCD was low in the context of ocular surface disease, a treatment to increase the conjunctival goblet cell count before artificial tear treatment was reported to contribute to the treatment of such diseases[21].

In our study, the conjunctival GCD was the highest in the control group compared to other groups and the lowest in the dry eye group compared to other groups. The goblet cell squamous metaplasia scores were the highest in the dry eye group compared to other groups and the lowest in the control group compared to other groups. This findings suggest that, dry eye disease may be the most severe form of ocular surface disease in our groups.

Using diagnostic tests in dry eye patients, Moore *et al*[22] established a relationship between low GCD and low TBUT values. In our study, we found a very good correlation between GCD and TBUT, and between the *Schirmer* test results. We believe that the evaluation of the grade of ocular surface staining with vital stains, conjunctival GCD, and Nelson metaplasia scores could be an effective method with which to characterise ocular surface health and the severity of ocular surface inflammation. Therefore, we think that ocular surface staining with vital stains and conjunctival impression cytology tests would be beneficial during follow-up treatment.

Kunert *et al*[23] reported that topical cyclosporin use in dry eye patients increased GCD, and Moore *et al*[24] found that topical cyclosporine use raised mucin release in their model of dry eye. These publications and our results demonstrate that dry eye syndrome is inflammation-based and that this inflammation reduces mucin release by affecting each layer of the ocular surface, especially conjunctival goblet cells, and as a result, has an impact on the mucin layer of the tear film.

A study on conjunctival impression cytology and GCD in anterior blepharitis patients has yet to be reported in the literature. In a study conducted on GCD in ocular surface diseases, Nelson and Wright[15] reported low GCD when comparing the chronic blepharitis patients with the controls and a similar density in comparison to the dry eye group. In an experimental mouse model study, Yeh *et al*[25] reported lower GCD in the group with Meibomian gland dysfunction.

In our study, the conjunctival GCD was lower and the goblet cell squamous metaplasia scores were higher in the anterior blepharitis group compared to controls. In clinical applications, such changes in conjunctival goblet cells manifest as dry eye-like symptoms in blepharitis patients. We believe that these changes in goblet cells could be a reflection of chronic inflammation found in the pathophysiology of anterior blepharitis disease on the ocular surface.

In allergic conjunctivitis conditions, the degree and severity of the allergic reaction are quite inconsistent[6]. Regarding the correlation between allergic conjunctivitis and the ocular surface, different results were reported for various subtypes of the condition. Although our purpose is not to compare forms of allergic conjunctivitis, we discussed with the impression cytology studies related to allergic conjunctivitis conditions in the literature. Aragona *et al*[26] reported a significant increase in GCD compared with the control group in their study on conjunctival impression cytology in vernal conjunctivitis patients, these goblet cells were smaller in vernal

conjunctivitis group than controls. Similarly, in their conjunctival biopsy study in atopic keratoconjunctivitis patients, Roat *et al*[27] established increases in GCD and epithelial mitotic rate compared with the control group. These information suggest that; goblet cells may play a role in pathogenesis of vernal and atopic conjunctivitis. They think; in the first phase of the conjunctival response to inflammation GCD increase and in the late phase of the conjunctival response to inflammation GCD decrease.

In a study by Hu *et al*[28] on conjunctival impression cytology in atopic keratoconjunctivitis patients diagnosed with vernal conjunctivitis and atopic dermatitis, GCD was reported as lowest in the atopic group and lower in the vernal group compared to the control group. In this study, the squamous metaplasia stages of goblet cells were also examined, and the atopic and vernal conjunctivitis group received higher squamous metaplasia scores than the control group[28]. The authors relate the differences in conjunctival goblet cells in these types of allergic conjunctivitis with a more severe course of inflammation. Allergic conjunctivitis manifestations that developed in atopic dermatitis patients caused more serious ocular surface problems.

In a conjunctival impression cytology study by Toda *et al*[18] among allergic conjunctivitis patients, GCD was reported as low. Similarly, GCD was established as low in an experimental allergic conjunctivitis model study by Merayo-Lloves *et al*[29]. In an experimental allergic conjunctivitis mouse model by Kunert *et al*[30], a decrease in GCD and mucin gene expression was reported after repeated encounters with allergens. The authors also reported an increase in GCD right after the encounter was terminated and no significant difference in GCD or mucin release 48h after the encounter when compared with the control group.

In a study by Dogru *et al*[16] on topical olopatadine treatment in allergic conjunctivitis patients, GCD was lower in conjunctival impression cytology specimens taken before commencing the anti-allergic treatment when compared with the control group. Goblet cell squamous metaplasia scores evaluated as part of the impression cytology were higher in patients than in the control group. The same study found an increase in GCD and a decrease in squamous metaplasia scores with anti-allergic treatment.

In our study, when comparing the seasonal allergic conjunctivitis group with the control group, the conjunctival GCD was lower and the goblet cell metaplasia scores were higher. We believe that the inflammatory response stimulated by toxic effects of allergens results in ocular surface damage that affects conjunctival goblet cells, resulting in reduced density and metaplastic cell morphology. Such changes in conjunctival goblet cells occur as dry eye-like symptoms in seasonal allergic conjunctivitis patients. We believe that such a decrease in GCD is a reflection of the role of chronic inflammation in the pathophysiology of this disease as reflected on the conjunctiva and the ocular surface.

These three diseases involve inflammation that causes damage parallel to the severity of local inflammation on the ocular surface. This damage has been established to have serious effects, especially on conjunctival GCD and goblet cell metaplasia. We believe that such a loss in goblet cell density and the severe degree of associated metaplasia are correlated with patient symptoms and clinical findings.

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