

Comparison of Tear Film Profile, Conjunctival Impression Cytology, and Conjunctival Biopsy in Patients with Dry Eye

Sunandan Sood,¹ Raman Shukla,² Manisha Nada,² Ashok Kumar Khurana,² Brijbala Arora³

¹Department of Ophthalmology, Government Medical College, Chandigarh, ²Department of Ophthalmology, and ³Department of Pathology, Pandit BD Sharma Post Graduate Institute of Medical Sciences, Rohtak, Haryana, India

Aim: To carry out a comparative study of 3 different methods for assessing severity of disease in patients with dry eye: tear film profile, conjunctival impression cytology, and conjunctival biopsy

Patients and Methods: Fifty patients with clinically diagnosed dry eye underwent 6 tests for tear film adequacy, followed by conjunctival impression cytology and histopathological examination of a conjunctival biopsy.

Results: The clinical severity of dry eye categorised by tear film profile tests as mild, moderate, or severe correlated with the grading of conjunctival impression cytology in 98% of cases and conjunctival biopsy in 90% of cases.

Conclusions: The comprehensive tear film profile method used in the present study is a reliable indirect indicator of cytological changes in the conjunctiva in patients with dry eye. Conjunctival impression cytology, a non-invasive technique, seems to be a more suitable option than conjunctival biopsy when direct documentation of cellular changes is required.

Key Words: Biopsy, Conjunctiva, Cytology, Diagnostic techniques and procedures, Dry eye syndromes

Asian J Ophthalmol 2006;8(1):24-27

Introduction

Dry eye refers to a state of tear film instability that is incited and later established as a result of a deficiency of components of the tear film and/or an insufficient interaction between the mucin layer and cell surface glycoproteins.¹ Clinical examination and tear film tests such as the Schirmer-1 test, tear film break-up time test, marginal tear strip assessment, and fluorescein, rose bengal, or lissamine green staining are considered sufficient to diagnose and grade severity of dry eye. Conjunctival biopsy has

been recommended for establishing dry eye histopathologically.² Conjunctival impression cytology (CIC), a non-invasive technique in which conjunctival impressions are taken to examine cellular structure, has also been used for diagnosis of dry eye.

Conjunctival biopsy is time-consuming and cumbersome to perform and may not be accepted by patients. Furthermore, in clinical practice, CIC may not be feasible in all centres. Therefore, in the present study, assessments of individual patients by tear film profile tests, CIC, and conjunctival

biopsy were compared to determine whether the tear film profile accurately predicts morphological changes in the conjunctiva.

Patients and Methods

Patients were enrolled on the basis of symptoms suggestive of dry eye: foreign body sensation (sandy), irritation, smarting, excessive secretions, itching, eye 'dryness', non-specific ocular discomfort, chronically 'sore' eyes that did not respond to a variety of treatment modalities (with or without associated symptoms of redness), photophobia, and blurred vision. A detailed ocular examination including slit-lamp biomicroscopy was performed. Clinically, dry eye was suspected if patients had the following symptoms: stringy mucus, particulate matter in the tear film, lustreless ocular surface, conjunctival xerosis, Bitot's spots, papillary conjunctivitis, or filamentary keratitis. Systemic complaints in patients with established dry eye such as dry mouth or throat, difficulty in swallowing, painful joints or joint stiffness, or myalgia were noted if present.

The tear film profile was determined for each patient as described in detail elsewhere.³ Tests were carried out in the following sequence: tear film break-up time (mean of 3 readings);⁴ examination of the marginal tear strip;² examination of cornea for fluorescein staining; the Schirmer-1 test, performed under local anaesthesia;⁵ assessment of rose bengal 1% staining to detect mucus and stained areas using diffuse white light;⁶ and lissamine green staining, performed by placing an autoclaved strip in the inferior fornix for 1 minute. A slit lamp was used for the tear film break-up test and for assessing staining with the various dyes. Scores were assigned for each test and the total score was used as a measure of severity of disease as shown in Table 1.

Fifty patients, 21 men and 29 women (mean age, 48.8 years), with clinical

Table 1. Scoring system for diagnosis and grading of severity of dry eye based on tear function tests.³

Tear function test	Score*			
	0	1	2	3
Break-up time (seconds)	>10	6.1-10	3.1-6	Absent
Marginal tear strip	Intact	Scanty	Markedly diminished or discontinuous	Absent
Fluorescein staining	Absent	Fine punctate	Coarse punctate	Diffuse
Schirmer-1 test (mm/5 minutes)	>10	5-10	3-4	0-2
Rose bengal score	0-3	4-5	6-7	8-9
Lissamine green score	0-3	4-5	6-7	8-9

* Total score for all tests indicates disease severity: 0-1 = no dry eye; 2 = suspected dry eye; 3-8 = mild dry eye; 9-13 = moderate dry eye; 14-18 = severe dry eye.

Table 2. Scoring system for assessing patients with dry eye by conjunctival impression cytology.⁸

Stage*	Cytological features (nuclear/cytoplasmic ratio)	Severity of disease
0	No keratinisation; moderate number of goblet cells; uniform, non-goblet epithelial cells with blue-green cytoplasm (1:1)	Normal
1	No keratinisation; decreased goblet cell density; mild enlargement of non-goblet epithelial cells with blue-green cytoplasm (1:2 to 1:3)	Mild
2	No keratinisation; total loss of goblet cells; all epithelial cells flattened, moderately enlarged and cytoplasm slightly pink (1:4)	Mild
3	Early mild keratinisation; keratin filaments present; no goblet cells; squamous cells with metachromatic change of cytoplasm to pinkish colour (1:6)	Moderate
4	Moderate keratinisation; densely packed keratin filaments; no goblet cells; large, squamoid, metachromatic epithelial cells (1:8)	Moderate
5	Advanced keratinisation; dense keratin filaments; pyknotic nuclei; no goblet cells; more keratinised cells with shrunken cytoplasm	Severe

symptoms of dry eye and a tear film profile indicative of mild to severe dry eye underwent further investigation. Under topical anaesthesia (lignocaine hydrochloride 1%), CIC was carried out in one eye as described by Wittpen et al⁷ and the resulting slides were examined microscopically and graded according to stages defined by Tseng.⁸ Stages 1 and 2 were interpreted as mild, stages 3 and 4 as moderate, and stage 5 as severe dry eye (Table 2). After informed consent, a conjunctival biopsy was taken from the same eye from the upper bulbar conjunctiva approximately 5 mm from the limbus. The tissue was processed, sectioned, and stained with haematoxylin and eosin.^{9,10} Scores were assigned for changes in the density of goblet cells and the degree of stratification of epithelial cells described by Abdel-Khalek et al^{9,10} and the total score was used to assess the severity of dry eye (Table 3).

Results

Based on the results of the tear film profile, dry eye was categorised as mild in 25 patients, moderate in 22 patients, and severe in 3 patients (Table 4). When assessed by CIC according to the criteria of Table 2, twenty four patients were categorised as mild, 23 as moderate, and 3 as severe dry eye (Table 4). For 98% of patients, the assessment of severity based on CIC matched that obtained using the tear film profile.

Grading of histopathological changes typical of dry eye using a scoring system based on the data of Abdel-Khalek et al^{9,10}

indicated that 2 patients had no dry eye, 23 patients had mild dry eye, and 25 had moderate to severe dry eye (Table 4). In 90% of patients, the severity of dry eye based on conjunctival biopsy matched that indicated by the tear film profile and CIC. However, 2 patients assessed as stage 1 by CIC, who had total tear film profile scores of 4 and 5, were assessed as having no dry eye by biopsy. This may indicate that conjunctival biopsy lacks sensitivity for detecting initial stages of dry eye or may be a reflection of the fact that biopsy samples are small and may not always accurately reflect the status of the conjunctiva as a whole. Further, conjunctival biopsy was unable to distinguish between 3 patients with CIC and tear film profiles indicative of severe dry eye and other patients assessed as having moderate dry eye by those methods, because histopathological changes were the same in all these patients.

Discussion

A number of attempts have been made to diagnose and classify the severity of dry eye on the basis of clinical symptoms and signs or one or more tear film profile tests.^{11,12} In the present study, a more comprehensive tear film profile assessment was used, in which scores from 6 tear film tests were combined to categorise patients with dry eye as mild, moderate, or severe.³

The importance of CIC in the diagnosis of dry eye has previously been highlighted.^{13,14} Attempts to use CIC with a conventional staining technique to grade dry eye severity failed to define the morphological changes of the conjunctival

Table 3. Scoring system for assessing patients with dry eye by conjunctival biopsy.^{9,10}

Goblet cell density	Score*	Epithelial stratification [†]	Score*
Normal (<10/mm)	0	25% stratified	0
Scanty (3-9/mm)	1	25-50% stratified	1
Absent	2	50-75% stratified	2

* Total score indicates disease severity: 0 = normal; 1-2 = mild dry eye; 3-4 = moderate to severe dry eye.

[†] Includes superficial segment flattening and elongation, assessed over entire length of epithelium.

Table 4. Comparison of data obtained by 3 different methods for grading severity of dry eye.

Dry eye grading	Number of patients		
	Men (n = 21)	Women (n = 29)	Total (%)
Tear film profile			
Mild	11	14	25 (50)
Moderate	10	12	22 (44)
Severe	0	3	3 (6)
Conjunctival impression cytology			
Mild	11	13	24 (48)
Moderate	10	13	23 (46)
Severe	0	3	3 (6)
Conjunctival biopsy			
No dry eye	0	2	2 (4)
Mild	11	12	23 (46)
Moderate to severe	10	15	25 (50)

epithelium in the moderate and advanced stages of squamous metaplasia, particularly when goblet cells were absent.¹³ This problem was overcome by Tseng who, using Gill's modified Papanicolaou stain, succeeded in defining 5 different stages of squamous metaplasia of conjunctival epithelium, based on goblet cell density and increasing cellular stratification and keratinisation.⁸ In the present study, in which stages 1 and 2 were classified as mild, stages 3 and 4 as moderate, and stage 5 as severe dry eye, there was very close agreement between CIC and the tear film profile method. Thus, the present study substantiates earlier observations^{14,15} that CIC is a simple, non-invasive, reproducible, and sensitive technique, useful not only for establishing the diagnosis of dry eye but also for grading its severity.

There are very few reports of conjunctival biopsy carried out in dry eye states.^{9,10,15} In one study, using histopathological assessment of goblet cell density as the sole criterion for diagnosis was found to be inadequate.¹⁶ Abdel-Khalek et al have recommended that 2 parameters, goblet cell density and epithelial stratification, be used not only for diagnosis but also for assessing the severity of dry eye.^{9,10} Using the system of Abdel-Khalek et al as a basis, a simple scoring system was developed for use in the present study. Assessments made in this

way showed reasonable agreement with assessments obtained by CIC or the tear film profile. However, 2 patients diagnosed with mild dry eye by the latter 2 methods showed no histopathological abnormalities. In addition, conjunctival biopsy was not able to discriminate between patients with moderate and severe dry eye.

There have been some reports of comparisons of tear film profile and CIC^{13,14} and CIC and conjunctival biopsy¹⁵ in dry eye. However, the present study appears to be the first in which patients have been assessed by all 3 methods. In the present study, almost 100% agreement was achieved between assessments made by the tear film profile³ and a CIC grading system based on the stages of Tseng.⁸ This is consistent with the results of a previous study.¹⁴ However, in the latter study a different staging system was used, in which the presence of some goblet cells was observed even in advanced epithelial stratification.¹⁴ Another study has indicated a good correlation between a value for Schirmer's test of less than 5.5 mm/5 minutes and CIC in patients with keratoconjunctivitis sicca.¹⁷

In the present study, 90% of the patients categorised as having mild and moderate dry eye on the basis of conjunctival biopsy were placed in the same categories by tear film profile testing and CIC. Others have reported a 100% correlation between

assessments made by CIC and conjunctival biopsy.¹⁵

Previous studies have established that CIC is of immense value for diagnosing the various grades of severity of dry eye and gives results that correlate very well with the results of tear film profile tests. It is simple, rapid, non-invasive, reliable, and reproducible and thus has been recommended for early diagnosis of dry eye states. Conjunctival biopsy, on the other hand, is regarded as very reliable for diagnosing dry eye but unsatisfactory for establishing the severity of disease.

The present study indicates that it is sufficient to carry out tear film profile tests according to the method of Khurana et al³ to grade the severity of dry eye. It also indicates that, when documentation of cellular changes is needed, CIC should be regarded as preferable to conjunctival biopsy. It is a non-invasive and very sensitive method for both establishing the diagnosis of dry eye and assessing its severity.

References

1. Tseng SC. Topical tretinoin treatment for dry-eye disorders. *Int Ophthalmol Clin* 1987;27:47-53.
2. Whitcher JP. Clinical diagnosis of the dry eye. *Int Ophthalmol Clin* 1987;27:7-24.
3. Khurana AK, Chowdhry R, Ahluwalia BK, Gupta S. Tear film profile in dry eye. *Acta Ophthalmol (Copenh)* 1991;69:79-86.
4. Lemp MA, Dohlman CH, Kuwabara T. Dry eye secondary to mucus deficiency. *Trans Am Acad Ophthalmol Otolaryngol* 1971;75:1223-1227.
5. Prause JU. Tears absorption into the filter-paper strip used in the Schirmer-I test. A methodological study on a critical survey. *Acta Ophthalmol (Copenh)* 1982;60:70-78.
6. Van Bijsterveld OP. Diagnostic tests in the sicca syndrome. *Arch Ophthalmol* 1969;82:10-14.
7. Wittpen JR, Tseng S, Sommer A. Detection of early xerophthalmia by impression cytology. *Arch Ophthalmol* 1986;104:237-239.
8. Tseng SC. Staging of conjunctival squamous metaplasia by impression cytology. *Ophthalmology* 1985;92:728-733.
9. Abdel-Khalek LM, Williamson J, Lee WR. Morphological changes in human conjunctival epithelium. I. In normal elderly

- population. *Br J Ophthalmol* 1978;62:792-799.
10. Abdel-Khalek LM, Williamson J, Lee WR. Morphological changes in human conjunctival epithelium. II. In keratoconjunctivitis sicca. *Br J Ophthalmol* 1978;62:800-806.
 11. Manthorpe R, Andersen V, Jensen OA, Oxholm P, Prause JU, Schiødt M. Editorial comments to the four sets of criteria for Sjögren's syndrome. *Scand J Rheumatol Suppl* 1986;61:31-35.
 12. Franck C. Eye symptoms and signs in buildings with indoor climate problems ('office eye syndrome'). *Acta Ophthalmol (Copenh)* 1986;64:306-311.
 13. Nelson JD, Havener VR, Cameron JD. Cellulose acetate impressions of the ocular surface. Dry eye states. *Arch Ophthalmol* 1983;101:1869-1872.
 14. Petroustos G, Paschides CA, Karakostas KY, Psilas K. Diagnostic tests for dry eye disease in normals and dry eye patients with and without Sjogren's syndrome. *Ophthalmic Res* 1992;24:326-331.
 15. Reddy M, Reddy PR, Reddy SC. Conjunctival impression cytology in dry eye states. *Indian J Ophthalmol* 1991;39:22-24.
 16. Ralph RA. Conjunctival goblet cell density in normal subjects and in dry eye syndromes. *Invest Ophthalmol* 1975;14:299-302.
 17. Rolando M, Terragna F, Giordano G, Calabria G. Conjunctival surface damage distribution in keratoconjunctivitis sicca. An impression cytology study. *Ophthalmologica* 1990;200:176-178.

Address for Correspondence

Dr Manisha Nada
 22/9J, Medical Campus
 Post Graduate Institute of Medical Sciences
 Rohtak, Haryana 124001
 India
 Tel: (91 1262) 213 458
 Fax: (91 1262) 211 308
 E-mail: sanjeevparshad@rediffmail.com

SEAGIG Membership

To join SEAGIG, please visit the SEAGIG website at www.seagig.org. Membership categories are as follows:

- Glaucoma Member — any ophthalmologist in active practice who has completed a Glaucoma Fellowship and/or whose work consists of at least 50% glaucoma — US\$60
- Ophthalmic Member — any doctor working as an ophthalmologist in the country of residence of that doctor — US\$50
- Trainee Member — any medical practitioner participating in an ophthalmic vocational training programme — US\$40
- Medical Member — any medical practitioner with an interest in glaucoma — US\$20
- Research Member — any person who is a vision scientist — US\$20
- Associate Member — any person who is an eye care health worker who is not a medical practitioner — US\$20

Glaucoma Members, Ophthalmic Members, and Trainee Members will receive *Asian Journal of OPHTHALMOLOGY* free as part of their membership. Medical Members, Research Members, and Associate Members may receive *Asian Journal of OPHTHALMOLOGY* for an annual subscription fee of US\$30. All SEAGIG members receive a 1-year online subscription to *Asian Journal of OPHTHALMOLOGY*, access to the members-only parts of the SEAGIG website, a copy of the **Asia Pacific Glaucoma Guidelines**, and a **10% discount on the attendance rates of SEAGIG and AOGS conferences** (see the Bulletin Board for details of these forthcoming conferences). Membership runs for 1 year from the date of application.

Free Additional Year of SEAGIG Membership!

Full SEAGIG members (any of the categories above) will receive an additional year of membership for free. All current members and new members joining in 2005 will receive 1 full year's membership for free from the date of expiry of their paid membership. Full SEAGIG membership rights will apply.

Free Online SEAGIG Membership Trial

Registered ophthalmologists who are not yet full SEAGIG members can try out the SEAGIG website before enrolling as full members. SEAGIG is offering 1-year free online membership from now until 31 December 2006. Free online trial SEAGIG membership provides the following for 1 calendar year from the date of application:

- free access to the SEAGIG website (www.seagig.org), including access to all members-only sections of the website
- free online access to *Asian Journal of OPHTHALMOLOGY*
- free online access to the *Asia Pacific Glaucoma Guidelines*.*

* Free online trial SEAGIG membership does not provide SEAGIG Board standing or voting rights, a 10% discount for registration at SEAGIG or AOGS conferences, hard-copy subscription to *Asian Journal of OPHTHALMOLOGY*, or a hard copy of the *Asia Pacific Glaucoma Guidelines*.

To apply for 1-year free online membership, please visit the SEAGIG website at www.seagig.org.