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Corneal Epithelial Opacity in Dysfunctional Tear Syndrome

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Abstract

Purpose—To compare the appearance of the superficial corneal epithelium in patients with dysfunctional tear syndrome (DTS) and an asymptomatic control group using laser scanning confocal microscopy and determine the correlations between confocal microscopic findings and clinical severity parameters.

Design—Prospective case control study.

Methods—Thirty one patients with newly diagnosed DTS and 21 asymptomatic control subjects were evaluated for this study. Subjects with DTS were classified into four levels of clinical severity (DTS 1–4) based on the Delphi dry eye panel report criteria. The Heidelberg Retina Tomograph 2 Rostock Cornea Module (HRT2-RCM) laser scanning confocal microscope was used to image the superficial corneal epithelium. Areas of single or multiple opaque superficial epithelial cells were measured as a percentage of the $400 \times 400 \mu\text{m}$ field area in four randomly selected confocal images from each eye. Spearman correlations between the confocal findings and severity of symptoms, visual acuity and ocular surface signs were calculated.

Results—The mean area of opaque superficial corneal epithelial cells was significantly greater in DTS patients than normal subjects ($p < 0.0001$). Significant differences were observed between the DTS severity groups and the control group ($p < 0.001$), except for the DTS 1 group. The area of opaque cells significantly increased with level of clinical severity. The confocal findings showed significant correlation with clinical severity parameters, including blurred vision symptoms ($r = 0.86$, $p = 0.0001$), best corrected visual acuity (BCVA) (Spearman $r = 0.4$, $p = 0.03$), conjunctival lissamine green staining scores (Spearman $r = 0.4$, $p = 0.026$), corneal fluorescein staining scores (Spearman $r = 0.5$, $p = 0.002$) and videokeratoscopic surface regularity index (Spearman $r = 0.5$, $p = 0.02$).

Conclusion—Morphological changes in the superficial corneal epithelium of DTS patients detected by laser scanning confocal microscopy correlates with blurred vision symptoms and objective severity parameters. Objective confocal image analysis of the superficial corneal epithelium may prove useful for classifying DTS severity and for monitoring the efficacy of therapies.

Introduction

Dry eye syndrome is a prevalent condition affecting 14% to 33% of the population worldwide.^{1–3} Traditionally, dry eye has been classified into conditions with hyposecretion and/or increased evaporation of tears. Dysfunctional tear syndrome (DTS) was proposed by the Delphi

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panel report in 2006 as a more encompassing term for dry eye with the rationale that inflammatory mechanisms are involved in the pathophysiology of the disease.⁴ It is well recognised that DTS causes disease of the superficial corneal epithelium that includes deranged corneal epithelial barrier function, punctate epithelial erosions and surface irregularity.^{5–6} Previously reported studies using confocal microscopy to evaluate the corneal epithelium in DTS have found decreased density of superficial epithelial cells and irregular, patchy opaque superficial epithelial cells.^{7–10} The purpose of our study was to determine if there was a difference in the area of opaque superficial corneal epithelial cells in confocal microscopic images between an asymptomatic control group and DTS patients. Furthermore, the correlations between the area of opaque superficial corneal cells and severity of subjective symptoms and objective clinical parameters of DTS were determined.

Materials and Methods

Patients

Thirty-one patients with DTS meeting the inclusion and exclusion criteria were enrolled for the study at the Ocular Surface Center at Baylor College of Medicine, Houston, Texas. Patients completed the Ocular Surface Disease Index (OSDI) symptom severity questionnaire, followed by a complete ocular surface examination of both eyes by one of the investigators (S.C.P) in the following sequence: visual acuity measured with an ETDRS chart under standard mesopic conditions, Tomey TMS-2N computerized videokeratoscopy (CVK) measuring the Klyce Surface Regularity Index (SRI), biomicroscopic examination of the lid margins and meibomian glands, fluorescein tear break-up time (TBUT), corneal fluorescein staining, conjunctival lissamine green staining, Schirmer I test and confocal microscopic examination of the corneal surface with the Heidelberg Retina Tomograph 2 Rostock Cornea Module (HRT2-RCM).

Fluorescein Tear Break-up Time (TBUT)

The TBUT was evaluated 2 minutes after the inferotemporal bulbar conjunctiva was touched with a sodium fluorescein strip (Fluor-I- strip, Bausch & Lomb Pharmaceuticals Inc, Tampa, Florida, USA) wet with preservative- free saline (Unisol; Alcon, Fort Worth, Texas, USA). Subjects were instructed to blink, and the precorneal tear film was examined under blue-light illumination with a biomicroscope and x10 objective. The interval between the blink and the appearance of the first dark spot or discontinuity in the precorneal fluorescein-stained tear layer was then recorded. Three separate readings were taken for each eye, and the results were averaged.¹¹

Conjunctival Lissamine Green Staining

The inferotemporal bulbar conjunctiva was touched with a lissamine green strip (Alpha med, Ocusoft, Richmond, Texas) wet with preservative- free saline (Unisol; Alcon, Fort Worth, Texas, USA). The intensity of conjunctival lissamine green staining was recorded using a modified van Bijsterveld grading scheme on a scale of 0 (none) to 3 (confluent) in the nasal conjunctiva and temporal conjunctiva.²¹

Corneal Fluorescein Staining

The ocular surface was examined two minutes after fluorescein instillation into the tear film as described above. The Baylor grading scheme was used to grade the intensity of corneal fluorescein staining in five different zones of the cornea (central, superior, temporal, inferior, and nasal) based on the number of dots on a 5-point scale (no dot = 0; 1–5 dots = 1; 6–15 dots = 2; 16–30 dots = 3; more than 30 dots = 4; if there is one area of confluence add 1; two or more areas of confluence add 2; filamentary keratitis add 2).¹¹

Inclusion criteria for DTS included a symptom severity score >20 and TBUT ≤ 7 seconds. Meibomian gland disease (MGD) was diagnosed by evidence by dysfunction (lack of expressible meibum from $\geq 75\%$ of glands) and the presence of two or more morphological changes of the meibomian glands, including acinar atrophy, orifice metaplasia, and vascular dilation on the posterior lid margin.¹² Patients were excluded if they were using any topical medications other than non-preserved artificial tears, contact lens, had ocular surgery in the past year or had evidence of other ocular surface or retinal diseases. The United States European Study Group consensus criteria were used for diagnosis of Sjögren syndrome and Stevens-Johnson syndrome was diagnosed in patients with a history of acute vesiculobullous cutaneous eruption with involvement of two or more mucus membranes, including the conjunctiva.¹³

The patients were stratified into four levels of clinical severity based on the signs and symptoms using the Dry Eye Workshop (DEWS) criteria, previously used by our group (Table 1).^{12,14,22} When not all criteria of a severity group were met, severity grading was based on the worst parameter. Twenty one normal control subjects were recruited as controls. Inclusion criteria for the normal subjects were absence of corneal and conjunctival dye staining and a symptom severity score ≤ 20 .

Confocal Microscopy

Image Acquisition—Confocal microscopy of the cornea was performed with the Heidelberg Retina Tomograph 2 Rostock Cornea Module (Heidelberg Engineering GmbH, Germany). The HRT2-RCM uses a 60x water-immersion lens (Olympus Europea GmbH, Hamburg, Germany) and a 670-nm diode laser as the light source with an observation area of $400\mu\text{m} \times 400\mu\text{m}$ in each digital image.¹⁵ After application of topical anesthesia (Proparacaine 0.5 %, Alcon, Fort Worth, Texas) to the lower conjunctival fornix, the patient's eye was positioned so the microscope objective was at the level of the corneal apex. A drop of optical coupling medium gel (GenTeal, 0.3% hypromellose; Novartis Ophthalmics, Basel, Switzerland) was then applied to the microscope objective and a single-use contact element (Tomocap) was placed on the microscope objective. The microscope objective with the contact element was slowly advanced towards the cornea until the corneal surface was gently touched. When the first superficial cells were seen, the digital micrometer gauge was set to zero, and the images were recorded. Sequential imaging was performed from the level of the corneal superficial epithelial layer posteriorly to the basal epithelial-Bowman's layer interface.

Image Analysis—Four images which had visible superficial epithelial cells were randomly selected for analysis. Previous reports of confocal microscopy of the corneal epithelium have noted indistinct cell outlines and overlapping nuclei in the superficial layer.¹⁶ Therefore, it is difficult to measure indices such as cell density per mm^2 or average cell area in μm^2 in the superficial epithelium. Several reports using confocal microscopy in dry eye patients have noted irregular or patchy opaque cells in the superficial epithelium.^{7–10} In order to eliminate the need for cell counting in areas where most cell outlines are indistinct, the total area of opaque cells with pycnotic nuclei, as a percentage of the total $400 \times 400 \mu\text{m}$ field area in four randomly selected images of the superficial corneal epithelium of each eye (Figure 1) were measured using the freely available image analysis software (Image J, <http://rsb.info.nih.gov/ij/>).¹⁷

Statistical Analysis

Clinical parameters and confocal data in the five study groups (normal, entire DTS, DTS1, DTS2, DTS3, DTS4) were compared by analysis of variance (ANOVA) with Tukey post hoc testing (GraphPad Prism, La Jolla, California, USA). Best corrected logmar visual acuity (BCVA) measurements were used for statistical analysis. Correlations between age, clinical parameters (BCVA, symptom severity, TBUT, videokeratographic SRI, Schirmer 1,

conjunctival and corneal staining scores) and confocal data were determined using rank (Spearman) correlations. The mean value for both eyes was used for comparison of clinical parameters.

Results

Demographic and clinical data

The demographic data and clinical parameters for all DTS patients, and each of the four levels of DTS severity are presented in Table 2. The sub classification of DTS conditions by disease type is in Table 3. There were 80% females in the control group and their mean age was 44 ± 19 years. Corneal fluorescein staining was significantly greater in level 4 DTS compared to levels 1, 2 and 3 ($p < 0.001$). Conjunctival lissamine green staining was significantly greater in level 4 compared to levels 1, 2 and 3 ($p < 0.001$).

Confocal microscopy data

Representative images of the superficial corneal epithelium in the asymptomatic control and DTS level 3 groups are shown in Figure 2. In most cases, the opaque cells usually extended 2 – 3 layers deep from the surface in the areas of opacity. The mean area of opaque superficial epithelium (both eyes and the worst eye) in the control and all DTS groups is presented in Table 4. The mean area of opaque superficial epithelium in both eyes of the control group was $5,544.11 \pm 3330.4$ sq μm , representing $3.46 \pm 2.4\%$ of the entire area. In contrast, the mean area of opaque epithelium in both eyes of the entire DTS group was $23,322.4 \pm 15,365$ sq μm which was $14.7 \pm 11.3\%$ of the entire area. The mean area of opaque superficial epithelium in the worst eye of the control group was $5,686.23 \pm 3,469$ sq μm and $30,227 \pm 19170$ in the DTS group. The mean percentage area of opaque cells in the worst eye was $3.4 \pm 2.4\%$ in the control group, compared to $18.3 \pm 11.9\%$ in the entire DTS group. A significant difference in the mean opaque area of both eyes was noted between control and DTS eyes ($p < 0.0001$). The mean opaque epithelial area of both eyes of all DTS severity subgroups, except the DTS 1 group, was significantly greater than the control group ($p < 0.001$). The worst eye findings in all the DTS severity sub groups was significantly higher than the control group ($p < 0.001$). The area of opaque superficial epithelial cells significantly increased with level of clinical severity ($p < 0.01$).

Correlation Analyses

Correlation between age and corneal epithelial opacity—No correlation was found between age and area of corneal epithelial cell opacity for the control or DTS groups (Table 5).

Correlation between clinical parameters and corneal epithelial cell opacity—The rank (Spearman) correlation coefficients between area of epithelial opacity and clinical parameters are presented in Table 6. No correlation was found between the confocal findings and irritation symptom severity scores; however, a significant correlation was found between the area of opaque cells and the questions specifically pertaining to symptoms of blurred vision ($r = 0.86$, $p < 0.0001$), (Figure 3). The BVCA of patients showed significant correlation with the area of opaque epithelial cells ($r = 0.4$, $p = 0.034$), (Figure 4). A significant correlation was found between the area of opaque cells and the conjunctival staining scores ($r = 0.4$, $p = 0.026$) and corneal staining scores ($r = 0.5$, $p = 0.002$), (Figures 5 and 6). There was a significant correlation of the corneal epithelial changes and the videokeratoscopic SRI ($r = 0.5$, $p = 0.02$), (Figure 7). No correlation was found between the area opaque cells and TBUT or Schirmer 1 scores.

Discussion

Dysfunctional tear syndrome (DTS) is a very prevalent condition having a variety of clinical presentations that include eye irritation, increased sensitivity to air drafts and blurred vision.^{1–4} The ocular irritation in DTS is accompanied by an unstable tear film and corneal epithelial disease that includes deranged corneal epithelial barrier function.^{5–6} Experimental murine models have shown that the desiccating stress of dry eye can induce apoptosis, disruption of tight junction proteins and desquamation of the apical corneal epithelial cells.⁷ Previously reported studies using confocal microscopy to evaluate the morphology of superficial corneal epithelium in dry eye have noted an increase in irregular opaque cells.^{8–11} To our knowledge, none of the previous studies have measured the area of opaque superficial corneal epithelial cells and correlated this finding with clinical severity parameters.

We found the mean area of these opaque superficial corneal epithelial cells was significantly increased in DTS compared to the control group. In fact, this confocal finding was significantly higher in all the DTS groups except the mild DTS 1 group. Furthermore, the area of opaque cells significantly increased with level of clinical severity of DTS.

No correlation was observed between these superficial epithelial abnormalities and age. A significant positive correlation was noted between the mean area of opaque superficial corneal epithelial cells and clinical severity parameters, including blurred vision symptoms, best corrected visual acuity and conjunctival and corneal dye staining scores. The cause of the opacification of the superficial epithelium has not been established. It is possible that these cells become opaque due to increased production of cornified envelope precursor proteins which have been noted to increase in human and experimental murine dry eye.¹⁸ It is also possible these represent dead or detaching epithelial cells that may have altered membrane permeability to fluorescein dye. Clinically, fluorescein is often observed to stain a focal partial or full thickness area of the cornea; consistent with this finding is our observation that the opaque epithelial cells extend to a depth of several layers from the surface in the areas of opacity. The presence of these opaque corneal epithelial cells could explain the blurred and fluctuating vision symptoms that many patients with DTS complain of, as well as the reports of decreased visual contrast sensitivity in dry eye.^{19–21} These cells likely diminish the optical properties of the cornea due to surface irregularity and light scatter. This concept is consistent with our observed correlation between area of opacity and the surface regularity index (SRI) measured by videokeratoscopy. The SRI has been reported to show high correlation with visual acuity as well as the severity of punctuate corneal fluorescein staining.²²

Our findings show that quantifying the area of opaque superficial corneal epithelial cells may prove to be a useful indicator of DTS severity and provide evidence that an abnormal corneal epithelium is the cause for decreased vision. They may prove to be a sensitive, objective and reproducible parameter for monitoring the efficacy of DTS therapies.

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C. Contributions of Authors: Design of Study: (J.J.C, K.R, S.C.P)

Conduct of the Study: (J.J.C, K.R, S.C.P)

Interpretation of Data: (J.J.C, K.R, S.C.P)

Preparation, review, and approval of manuscript: (J.J.C, K.R, S.C.P)

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Biography

Stephen C. Pflugfelder, M.D joined the faculty of the Cullen Eye Institute of Baylor College of Medicine as a Professor and Director of the Ocular Surface Center in July 2000. He has published over 160 peer-reviewed articles and over 45 book chapters and monographs, primarily in the field of cornea diseases and surgery. His research interests include the role of inflammation in dry eye and corneal bioengineering.

Figure 1. Corneal epithelial opacity in dysfunctional tear syndrome and Asymptomatic Controls. The total area of opaque cells in the superficial epithelium, were outlined and measured in square microns using the Image J analysis software.

Figure 2.
(Left) Corneal epithelial opacity in superficial corneal epithelium of a 46 year old asymptomatic control female.(Right) corneal epithelial opacity in superficial corneal epithelium of a 44 year old female patient with level 3 dysfunctional tear syndrome.

Figure 3.

Severity of blurred vision symptoms versus area of corneal epithelial opacity in dysfunctional tear syndrome. Correlation between area of opaque superficial corneal epithelial cells in μm^2 and severity of blurred vision symptoms measured by ocular surface disease questionnaire

Figure 4. Best corrected visual acuity (BCVA) versus area of corneal epithelial opacity in dysfunctional tear syndrome. Correlation between area of opaque superficial corneal epithelial cells in μm^2 and BCVA (logmar units)

Figure 5. Conjunctival staining score versus corneal epithelial opacity in dysfunctional tear syndrome. Correlation between area of opaque superficial corneal epithelial cells in μm^2 and the severity of conjunctival lissamine green staining

Figure 7. Videokeratographic surface regularity index (SRI) versus corneal epithelial opacity in dysfunctional tear syndrome. Correlation between area of opaque superficial corneal epithelial cells in μm^2 and SRI.

TABLE 1**Severity Grading Criteria for Dysfunctional Tear Syndrome**

Group	Symptom Severity Score	Tear Break-up (seconds)	Conjunctival staining Score	Corneal Staining Score
No DTS	≤ 20	>7	0	0
DTS 1	> 20	≤ 7	≤ 3	≤ 2
DTS 2	>20	≤ 7	≥ 3	≤ 8
DTS 3	>20	≤ 7	≥ 3	>8 , including central cornea or filaments
DTS 4	>20	≤ 7	≥ 3	≥ 12

DTS = dysfunctional tear syndrome; DTS 1 to 4 = dysfunctional tear syndrome severity levels 1 to 4.

Symptom severity score measured by ocular surface disease index (OSDI) questionnaire

TABLE 2
Clinical Features of Dysfunctional Tear Syndrome Patients Stratified by Severity

Severity Level	N	Mean Age ± SD (years)	%Female	Symptom Severity Score	Schirmer 1 Score (mm)	Conjunctival Staining Score	Corneal Staining Score	BCVA (logmar)
1	3	54.7 ± 14.5	100	40.3 ± 8.5	19.7 ± 12.5	0	1.83 ± 0.28	0.14 ± 0.15
2	17	58.2 ± 14.4	94	37.9 ± 9.7	17.06 ± 11.4	1.76 ± 2.07	4.2 ± 1.9	0.14 ± 0.13
3	7	52.2 ± 7.8	100	43.6 ± 11.4	8.2 ± 5.6	2.64 ± 2.68	9.7 ± 0.7 P < 0.001 ^a	0.07 ± 0.08
4	4	50.2 ± 42.4	75	41.7 ± 6.0	5.7 ± 3.3	5.87 ± 0.3 P < 0.003 ^b	14.1 ± 1.2 P < 0.001 ^b	0.90 ± 0.87
All DTS	31	56 ± 15	93.5	39.8 ± 9.6	13.1 ± 9.8	2.3 ± 2.4	6.3 ± 3.5	0.24 ± 0.28

SD = standard deviation, BCVA = best corrected visual acuity

The mean score of both eyes was used for the Schirmer 1 test, corneal fluorescein staining, conjunctival staining scores and BCVA

Symptom severity score measured by ocular surface disease index (OSDI) questionnaire

^a vs levels 1 and 2

^b vs levels 1, 2 and 3

Table 3

Subclassification of Dysfunctional Tear Syndrome

DTS level	All DTS	MGD	SS	SJS	Non-SS/ATD
1	3	1	0	0	2
2	17	6	6	0	5
3	7	1	5	0	1
4	4	0	2	2	0
Total	31	8	13	2	8

DTS = dysfunctional tear syndrome; MGD= meibomian gland disease; SS= Sjögren syndrome; SJS = Stevens-Johnson syndrome; Non-SS/ATD = Non Sjögren syndrome aqueous tear deficiency

Table 4

Area of Opaque Superficial Corneal Epithelium in Dysfunctional Tear Syndrome and Control Patients

Group	Mean opaque area in both eyes (μm^2)	% opaque area in both eyes	Mean opaque area in worse eye (μm^2)	% opaque area in worse eye
No DTS	5,544.11 $\pm$ 3330.4	3.46 $\pm$ 2.4	5,686.23 $\pm$ 3469.7	3.4 $\pm$ 2.4
All DTS	23,322.4 $\pm$ 15,365.5 P < 0.001 ^a	14.7 $\pm$ 11.3	30,227.7 $\pm$ 19170 ^a P < 0.001 ^a	18.3 $\pm$ 11.9
DTS 1	11,772.0 $\pm$ 4901.1 P < 0.01 ^b	7.33 $\pm$ 3.06	15,780.7 $\pm$ 8700.2 P < 0.001 ^b	11.4 $\pm$ 6.5
DTS 2	17,462.7 $\pm$ 13,629.8 P < 0.005 ^a , P < 0.005 ^b	10.6 $\pm$ 6.2	21,764.8 $\pm$ 12,791.1 P < 0.005 ^{a,b}	11.5 $\pm$ 2.4
DTS 3	22,653.7 $\pm$ 15,521 P < 0.05 ^a , P < 0.01 ^b	14.14 $\pm$ 9.7	32027.3 $\pm$ 17415.5 P < 0.001 ^{a,b}	20.0 $\pm$ 8.2
DTS 4	53,803.3 $\pm$ 22,571.8 P < 0.001 ^a	33.62 $\pm$ 14.1	65,504.3 $\pm$ 27007.2 P < 0.001 ^a	40.9 $\pm$ 16.8

% opaque area indicates the percentage area of opaque cells in the total $400 \times 400 \text{sq } \mu\text{m}$ field area Worse eye indicates the eye with the greatest area of opaque cells noted by confocal microscopic imaging

^a vs no DTS control;

^b vs level 4

Table 5

Correlation between area of opaque cells and age in control and Dysfunctional Tear Syndrome groups

Group	Opaque cells Average both eyes		Opaque cells Worse eye	
	Spearman r	P value	Spearman r	P value
Control	−0.31	0.19	0.02	0.94
DTS	0.1	0.58	0.08	0.66

None of the values achieved statistical significance

Worse eye indicates the eye with more opaque area, noted by confocal microscopic imaging

Table 6

Correlation between opaque cells and clinical severity parameters in Dysfunctional Tear Syndrome

Clinical Parameter	Opaque cells Average both eyes		Opaque cells Average worse eye	
	Spearman r	P value	Spearman r	P value
Symptom Severity Score	0.01	0.93	0.27	0.38
Symptom Severity pertaining to blurred vision	0.86	0.0001	0.863	0.0001
BCVA	0.4	0.03	0.3	0.04
TBUT	-0.06	0.73	-0.05	0.7
Conjunctival Staining	0.4	0.026	0.38	0.03
Corneal Staining	0.5	0.0025	0.53	0.0018
Surface Regularity Index	0.5	0.02	0.49	0.021
Schirmer 1	-0.17	0.3593	-0.17	0.35

Significant correlations are in bold font

Symptom severity score measured by Ocular surface disease index (OSDI) questionnaire, BCVA = best corrected visual acuity

TBUT = tear break up time

Worse eye indicates the eye with more opaque area, noted by confocal microscopic imaging