Article

Matrix Metalloproteinase 9 Point-of-Care Immunoassay Result Predicts Response to Topical Cyclosporine Treatment in Dry Eye Disease

Jae Yong Park[1], Bum Gi Kim[1], Jae Suk Kim[1], and Je Hyung Hwang[1]

[1] Department of Ophthalmology, Sanggye Paik Hospital, Inje University College of Medicine, Seoul, Korea

Correspondence: Je Hyung Hwang, Department of Ophthalmology, Sanggye Paik Hospital, Inje University, 1342 Dongil-ro, Nowon-Gu, Seoul 139-707, Korea. e-mail: violentviolet15@daum.net

Purpose: We evaluate a matrix metalloproteinase-9 (MMP-9) point-of-care immuno-assay (InflammaDry) as a prognostic tool for topical cyclosporine treatment.

Methods: A total of 20 healthy subjects and 40 patients meeting >3 dry eye disease (DED) criteria (ocular surface disease index [OSDI] score ≥ 12, tear film breakup time [TBUT] ≤10 seconds, Schirmer I test result ≤10 mm/5 minutes, corneal staining ≥1) were included. DED patients were treated with topical cyclosporine ophthalmic emulsion 0.05% twice daily for 1 month. The InflammaDry test was used to grade MMP-9 levels in the tear film. Treatment response was monitored using the OSDI score, TBUT, and Schirmer, corneal staining, and InflammaDry tests.

Results: Of the eyes, 18 (22.5%) were negative, 29 (36.3%) trace-positive, 16 (20.0%) weak-positive, 11 (13.8%) positive, and six (7.5%) strong-positive for MMP-9 at baseline. MMP-9 levels correlated with OSDI ($P = 0.049$), TBUT ($P = 0.001$), corneal staining ($P = 0.002$), and Schirmer test ($P = 0.027$) results. MMP-9–positive patients displayed decreased post-treatment MMP-9 levels ($P = 0.001$) and corneal staining score ($P < 0.001$), improved OSDI score ($P < 0.001$), and increased TBUT ($P < 0.001$) and Schirmer ($P = 0.009$) test values.

Conclusions: Semiquantitative MMP-9 grading correlated well with DED symptoms and signs, and could be used to predict patient status and monitor treatment response. MMP-9–positive patients responded more favorably to topical cyclosporine than did MMP-9–negative patients. Thus, the InflammaDry test may inform decisions regarding initiating topical cyclosporine treatment.

Translational Relevance: Semiquantitative MMP-9 could be used to predict patient status and monitor treatment response.

Introduction

Dry eye disease (DED) is a multifactorial disease affecting the tears and ocular surface; it results in symptoms of discomfort, visual disturbance, and tear film instability. In DED, increased osmolarity and inflammation have important roles.[1] DED is a relatively common disease worldwide, affecting 5%–30% of the population aged ≥50 years, and significantly reduces the quality of life of these patients.[2] Despite its high prevalence, its clinical diagnosis and management remain difficult, likely due to its multifactorial etiology and varied pathophysiologic mechanisms. Symptoms alone are not adequate for the diagnosis of DED, as similar symptoms can present with various other ocular surface diseases. Associations between symptoms and clinical tests are weak, since only 57% of symptomatic patients showed objective signs of DED.[3]

Since inflammation is increasingly recognized as a fundamental component of the pathophysiology of DED, diverse inflammatory cytokines, released during the vicious cycle of DED, have been studied, including interleukin-1β (IL-1β), IL-6, tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), caspase 3, transglutaminase 2 (TG-2), and matrix metalloproteinases (MMPs), such as MMP-3 and MMP-9.[4–8] Among these cytokines, MMP-9 has a crucial role in initiation and progression of ocular surface dis-

ease.[9,10] Interactions between the various cytokines and MMP-9 trigger inflammatory pathways, such as the stress-activated protein kinase (SAPK) signaling cascade and nuclear factor-κB (NF-κB) pathways, resulting in escalating inflammation.[11–13] Furthermore, MMP-9 promotes corneal extracellular matrix degradation and epithelial cell loss.[14]

Recent technologic developments have allowed clinical ophthalmologists to assess MMP-9 levels from tear samples of DED patients easily with the InflammaDry assay (Rapid Pathogen Screening, Inc., Sarasota, FL). InflammaDry is a noninvasive, disposable point-of-care assay that identifies the presence of MMP-9 at levels >40 ng/ml. The test result is visible within 10 minutes, allowing simple, but accurate testing in clinical outpatient settings.

Several studies have evaluated the efficacy of InflammaDry as an aid to DED diagnosis. An early study conducted with InflammaDry showed 85% sensitivity and 95% specificity in the diagnosis of DED.[15] Another prospective clinical trial with 237 DED patients showed 81% positive agreement of InflammaDry test results for mild DED.[16] A recent study reported 40.4% positive InflammaDry results in 47 DED patients and demonstrated that the positive results correlated well with the ocular surface disease index (OSDI), tear film break-up time (TBUT), Schirmer test, conjunctival staining, and corneal staining.[17] It seems reasonable that the InflammaDry results may aid in DED diagnosis, but the reported sensitivity demonstrates considerable variations, suggesting the need for other diagnostic approaches with InflammaDry.

One study conducting anti-inflammatory treatment in DED reported that the laboratory measurement of MMP-9 decreased significantly after corticoid treatment, and that patient symptoms also improved.[8] Considering the involvement of MMP-9 in inflammation in DED, it is reasonable to hypothesize that the InflammaDry assay may aid in predicting response to anti-inflammatory treatment of DED. We evaluated the efficacy of the InflammaDry test as a prognostic tool for deciding on initiating topical cyclosporine treatment, an essential anti-inflammatory treatment for DED.

Methods

Study Design

This study comprised a retrospective chart review of DED patients and cross-sectional data acquisition of normal controls. Data were collected at the Department of Ophthalmology of Sanggye Paik Hospital from January 2017 to December 2017. Approval for data collection and analysis was obtained from the institutional review board of Inje University which waived the need to obtain informed consent from DED patients whose records were reviewed retrospectively. Written informed consent was obtained from every normal control subject after explanation of the detailed protocols of the study. This research adhered to the tenets set forth in the Declaration of Helsinki.

Subjects

Information regarding age and sex was gathered before performing clinical ophthalmic examinations. Patients at the first medical examination at our hospital complaining of DED symptoms were considered primarily as participants. Ethnicity of every subject was identical as Koreans. All subjects underwent detailed ophthalmic examinations, including slit-lamp examination, noncontact tonometry, and fundus examination. Exclusion criteria were any ocular pathologies other than cataract and DED found during the ophthalmic examination; any history of topical anti-inflammatory treatment including topical steroids, cyclosporine, and diquafosol; any history of systemic disease other than hypertension; patient taking any medicines that may affect tear production, such as beta-blockers, benzodiazepines, hormone drugs, or antihistamines; any history of ocular disease, such as ocular surface disease other than DED, vitreoretinal disease, uveitis, optic neuropathy including glaucoma, amblyopia, and strabismus; wearing contact lenses within the previous 6 months; and any previous ocular surgery before enrollment in this study.

For DED patients, those who met >3 of the following criteria were included in the study: OSDI score ≥12; TBUT ≤ 10 seconds; Schirmer I test result ≤10 mm/5 min; and corneal staining test result ≥ 1. For normal controls, those who met all four of the following criteria were included in the study: OSDI score <12; TBUT >10 seconds; Schirmer I test result >10 mm/5 min; and no corneal staining.

Assessment of Clinical Parameters

A single experienced investigator (JHH) performed ocular surface evaluation and measured clinical parameters, including OSDI, questionnaire survey, TBUT, Schirmer I test, and corneal staining scores

assessment. According to the dry eye severity grading scheme from the Dry Eye Workshop Study (DEWS) classification,[2] the patients were stratified into groups with severity level from 1 to 4.

OSDI is a global DED assessment tool consisting of 12 questions, each self-scored by the patient, from 0 to 4, according to subjective symptoms. The final OSDI score is calculated as follows: (sum of scores × 25) / (number of questions answered). The OSDI score ranges from 0 to 100, with higher grading scores signifying greater disabilities regarding DED. The score ranges represent the severity of the disease symptom: 0 to 12 is normal, 13 to 22 mild, 23 to 32 moderate, and 33 to 100 severe disease.

For TBUT assessment, a fluorescein dye (Haag-Streit, Koeniz, Switzerland) was applied to the eyes, and TBUT was measured using a slit-lamp under cobalt-blue light. The interval between the last blink and first appearance of dark spots in the tear film was measured under ×10 magnification. The average value of three TBUT measurements was used for analysis.

Standardized Schirmer strips (Eagle Vision, Memphis, TN) were placed in the lateral third of the lower eyelid without anesthesia. After 5 minutes, the length of the portion of strips that was wet was measured.

The cornea staining score was determined in accordance with the Sjögren's International Collaborative Clinical Alliance (SICCA) registry ocular examination protocol.[18] Corneal punctate epithelial erosions (PEEs) were enumerated and scored after staining the cornea with fluorescein. Corneal staining scores were assigned as: PEEs absent, 0; 1 to 5 PEEs, 1; 6 to 30 PEEs, 2; and >30 PEEs, 3. An additional point was added if: (1) PEE occurred in the central 4 mm diameter portion of the cornea, (2) 1 or more filaments was seen anywhere on the cornea, and (3) 1 or more patches of confluent staining, including linear stains, were found anywhere on the cornea. The maximum possible score for each cornea was 6.

MMP-9 Assessment

Visual, qualitative, in vitro detection of MMP-9 protein levels of human tears was performed with the InflammaDry assay. A sampling fleece was dabbed gently at multiple locations along the inside of the patient's lower eyelid, releasing the lid every 2 to 3 dabs to allow the patient to blink. After 6 to 8 dabs along the conjunctiva, the sampling fleece was rested against the conjunctiva for an additional 5 seconds for sufficient tear sampling. The sampling fleece was placed gently on the sample transfer window of the test cassette body, and the sample collector was pressed firmly to snap it stably to the cassette body. The buffer vial was opened to immerse the absorbent tip for 20 seconds. The protective cap was replaced, and the test kit was placed flat on a horizontal surface for 10 minutes.

After 10 minutes, the results window was evaluated to observe the MMP-9 levels in the tear film. A results window without a blue line was considered invalid and was excluded from the study, while one with a red line was considered positive. A results window without a red line was allowed to incubate for an additional 5 minutes before confirming the test results, and was considered negative when the red line remained absent.

For samples with positive results, we applied a semiquantitative method of grading the intensity of the InflammaDry test result using a grading index referring to the manufacturer's instructions, for a more detailed evaluation of MMP-9 levels in the tear film. The signal intensity of the test result increases proportionally to the increasing concentration of MMP-9 present in the sample. Therefore, a more vivid red line indicated a higher MMP-9 concentration in the tear film. The positive red line was compared with the grading index to classify the result as trace-positive, weak-positive, positive, and strong-positive. Three well-trained observers (JYP, BGK, and JHH) individually interpreted the InflammaDry grading result, masked to subject characteristics and clinical diagnosis. The result was confirmed when the interpretations of at least two of the observers were identical.

Treatment Protocol

Patients diagnosed with DED were treated with topical cyclosporine ophthalmic emulsion 0.05%, instilling one drop twice a day in each eye, approximately 12 hours apart, in accordance with international recommendations concerning the use of cyclosporine for treating DED.[19] Treatment response was evaluated after a month of treatment, including the OSDI questionnaire survey, TBUT, Schirmer I test, corneal staining scores, and InflammaDry test result.

Statistical Analysis

For statistical analysis, a linear-by-linear association test was applied to investigate the trend between MMP-9 levels and OSDI severity, TBUT, corneal staining scores, and Schirmer test results. Parameters between DED patients and healthy controls were

Table 1. Characteristics of the Eyes With DED Stratified by Dry Eye Severity Grading Scheme

Dry Eye Severity Level	1	2	3	4
Number of eyes	11	42	23	4
MMP-9 grade (positive/negative, Trace/weak/positive/strong)	5/6 (3/2/0/0)	35/7 (19/11/5/0)	18/5 (7/2/4/5)	4/0 (0/1/2/1)
OSDI	12.92 ± 6.82	24.12 ± 17.96	52.21 ± 21.11	55.21 ± 8.42
TBUT (seconds)	9.27 ± 3.38	5.12 ± 2.91	3.70 ± 2.20	2.25 ± 0.50
Corneal staining (score 0/1/2)	1.09 ± 0.30 (10/1/0)	1.36 ± 0.58 (29/11/2)	2.09 ± 0.73 (5/11/7)	3.00 ± 0.00 (0/0/4)
Schirmer test (mm/5 min)	19.55 ± 6.70	12.19 ± 7.32	7.70 ± 2.38	4.25 ± 0.96

compared with t-tests and Mann-Whitney U tests. To compare clinical parameters before and after treatment, paired t-tests and Wilcoxon signed-rank tests were conducted. The level of significance was set at $P < 0.05$. The data were analyzed with SPSS 22.0 (SPSS, Inc., Chicago, IL).

Results

Patient Demographics

A total of 40 DED patients (mean age, 60.15 ± 9.66 [range, 34–81] years; seven male, 33 female) and 20 healthy subjects (mean age, 55.80 ± 12.46 [37–68] years; four male, 16 female) were enrolled in the study. Of the 40 DED patients, 30 (mean age, 60.70 ± 9.70 [34–78] years; five male, 25 female) returned for a second visit after a month of treatment and completed the clinical assessment. Neither age nor sex differed significantly between the patient and subject groups ($P = 0.10$ for age, $P = 0.11$ for sex). The clinical characteristics of the eyes with DED stratified by dry eye severity grading scheme from the DEWS classification are described in Table 1.

MMP-9 and Clinical Parameters of DED Patients

Of the 80 eyes in the 40 DED patients, 62 (77.5%) tested positive for MMP-9 overall at baseline. More specifically, 18 eyes (22.5%) were negative, 29 (36.3%) trace-positive, 16 (20.0%) weak-positive, 11 (13.8%) positive, and six (7.5%) strong-positive for the InflammaDry test. Intraclass correlation coefficient among the three independent observers was 0.791 ($P < 0.001$; 95% confidence interval, 0.74-0.84). There were no cases in which all three observers disagreed.

Average OSDI score of the patients was 32.21 ± 23.02 (range, 0–93.75): nine patients (22.5%) were normal, while 11 patients (27.5%) had mild, five

(12.5%) moderate, and 15 (37.5%) severe disease. The increasing MMP-9 level was associated with higher OSDI score ($P = 0.049$; Table 2).

Mean TBUT of the DED patients was 5.14 ± 3.27 seconds (range, 2–13). TBUT was >10, 5 to 10, and <5 seconds in 11 (13.8%), 13 (16.3%), and 56 (70.0%) eyes, respectively. Statistical analysis demonstrated that higher MMP-9 levels were associated with decreased TBUT ($P = 0.001$; Table 2).

Average corneal staining score of the DED patients was 0.61 ± 0.76 (range, 0–2): 44 eyes (55.0%) did not show any PEEs and were scored as 0, whereas 23 (28.8%) were scored as 1, and 13 (16.3%) as 2. Increasing MMP-9 levels also were associated with higher corneal staining scores ($P = 0.002$; Table 2).

Mean Schirmer test value was 11.51 ± 7.16 mm/5 min (range, 2–30) in DED patients. The result was >10, 5 to 10, and <5 mm/5 min for 31 (38.8%), 35 (43.8%), and 14 (17.5%) eyes, respectively. A higher MMP-9 level was significantly associated with decreased Schirmer test values ($P = 0.027$; Table 2).

Clinical Parameters of Healthy Controls

Of the 40 eyes from the 20 healthy controls, three (7.5%) showed positive results overall, which was significantly different from the 77.5% positive rate from DED patients ($P < 0.001$). All three eyes were trace-positive for MMP-9. Most healthy controls did not have symptoms of DED, with an average OSDI score of 1.16 ± 2.94, which differed significantly from the OSDI score of DED patients ($P < 0.001$). Mean TBUT of the healthy controls was 12.45 ± 3.35 seconds (range, 3–17) and no control showed any corneal staining. TBUT and corneal staining results were significantly different from those of the DED patients (both $P < 0.001$). Average Schirmer test value in the controls was 17.05 ± 6.13 mm/5 min

Table 2. Percentage of the Eyes Corresponding to Each Clinical Parameter Subdivided by MMP- Grades

		MMP-9 Grade				
		Negative	Trace	Weak	Positive	Strong
OSDI	Normal	44.4% (8/18)	13.8% (4/29)	37.5% (6/16)	0% (0/11)	0% (0/6)
	Mild	11.1% (2/18)	41.4% (12/29)	18.8% (3/16)	27.3% (3/11)	33.3% (2/6)
	Moderate	0% (0/18)	20.7% (6/29)	12.5% (2/16)	18.2% (2/11)	0% (0/6)
	Severe	44.4% (8/18)	24.1% (7/29)	31.3% (5/16)	54.5% (6/11)	66.7% (4/6)
TBUT (seconds)	TBUT>10	27.8% (5/18)	17.2% (5/29)	6.3% (1/16)	0% (0/11)	0% (0/6)
	10≥TBUT>5	33.3% (6/18)	10.3% (3/29)	12.5% (2/16)	18.2% (2/11)	0% (0/6)
	5≥TBUT	38.9% (7/18)	72.4% (21/29)	81.3% (13/16)	81.8% (9/11)	100% (6/6)
Corneal staining Score	0	72.2% (13/18)	51.7% (15/29)	68.8% (11/16)	27.3% (3/11)	33.3% (2/6)
	1	16.7% (3/18)	44.8% (13/29)	25.0% (4/16)	18.2% (2/11)	16.7% (1/6)
	2	11.1% (2/18)	3.4% (1/29)	6.3% (1/16)	54.5% (6/11)	50.0% (3/6)
Schirmer test (mm/5 min)	Schirmer>10	38.9% (7/18)	48.3% (14/29)	43.8% (7/16)	27.3% (3/11)	0% (0/6)
	10≥Schirmer>5	50.0% (9/18)	41.4% (12/29)	31.3% (5/16)	54.5% (6/11)	50.0% (3/3)
	5≥Schirmer	11.1% (2/18)	10.3% (3/29)	25.0% (4/16)	18.2% (2/11)	50.0% (3/3)

(range, 8–28), which was significantly different from that of the DED patients ($P < 0.001$).

MMP-9 Positivity and Treatment Response

Of the 60 eyes from 30 DED patients followed after a month of topical cyclosporine treatment, 46 (76.7%) were MMP-9 positive overall and 14 (23.3%) were MMP-9 negative. More specifically, of the MMP-9 positive eyes, 16 (34.8%) were trace-positive, 13 (28.3%) weak-positive, 11 (23.9%) positive, and six (13.0%) strong-positive (Table 3). After a month of treatment, the MMP-9 levels showed an overall decrease, with 13 eyes (28.3%) negative, 14 (30.4%) trace-positive, nine (19.6%) weak-positive, 10 (21.7%) positive, and none strong-positive. MMP-9 levels before and after the treatment were significantly different ($P = 0.001$).

Patient symptoms also were improved by a month of cyclosporine treatment (mean OSDI score 35.13 ± 19.99 and 25.00 ± 20.28 before and after treatment, respectively; $P < 0.001$). Furthermore, other clinical parameters showed general improvement. TBUT increased from 3.80 ± 2.09 to 5.57 ± 2.34 seconds ($P < 0.001$), corneal staining score decreased from 0.74 ± 0.80 to 0.09 ± 0.35 ($P < 0.001$), and Schirmer test values increased from 11.57 ± 7.62 to 13.46 ± 6.15 mm/5 min ($P = 0.009$).

The subdivided group of high MMP-9–positive eyes (positive and strong-positive MMP-9) showed consistent results, with overall significant improvement in MMP-9 grade ($P = 0.001$), OSDI score ($P = 0.001$), TBUT ($P < 0.001$), corneal staining ($P = 0.002$), and Schirmer test ($P = 0.015$; Table 4).

However, for MMP-9–negative eyes, most clinical parameters did not show improvements after cyclosporine treatment (Table 5). Furthermore, MMP-9 levels even worsened after therapy ($P = 0.010$). Of the 14 MMP-9–negative eyes, eight eyes became MMP-9–positive after the treatment. After a month of topical cyclosporine use, six eyes (42.9%) were MMP-9 negative, one (7.1%) trace-positive, five (35.7%) weak-positive, one (7.1%) positive, and one (7.1%) strong-positive. Average OSDI score was 36.72 ± 36.03 before and 37.46 ± 34.20 after treatment, with

Table 3. Topical Cyclosporine Treatment Response in MMP-9–Positive DED Patients

Characteristics	Before Treatment	After Treatment	P Value
MMP-9 grade (positive/negative, trace/weak/positive/strong)	46/0 (16/13/11/6)	33/13 (14/9/10/0)	0.001
OSDI	35.13 ± 19.99	25.00 ± 20.28	<0.001
TBUT (seconds)	3.80 ± 2.09	5.57 ± 2.34	<0.001
Corneal staining (score 0/1/2)	0.74 ± 0.80 (22/14/10)	0.09 ± 0.35 (43/2/1)	<0.001
Schirmer test (mm/5 min)	11.57 ± 7.62	13.46 ± 6.15	0.009

Table 4. Topical Cyclosporine Treatment Response in High MMP-9–Positive (Positive And Strong Positive) DED Patients

Characteristics	Before Treatment	After Treatment	P Value
MMP-9 grade (positive/negative, trace/weak/positive/strong)	17/0 (0/0/11/6)	(12/5) 5/4/3/0	0.001
OSDI	39.27 ± 20.16	24.70 ± 21.14	0.001
TBUT (seconds)	3.06 ± 1.44	4.65 ± 1.62	<0.001
Corneal staining (score 0/1/2)	0.74 ± 0.80 (5/3/9)	0.09 ± 0.35 (15/1/1)	0.002
Schirmer test (mm/5 min)	9.35 ± 6.21	12.00 ± 5.85	0.015

no significant difference ($P = 0.561$). Mean TBUT was 5.79 ± 2.86 and 6.57 ± 2.17, respectively ($P = 0.209$), and mean corneal staining score was 0.29 ± 0.61 and 0.14 ± 0.36, respectively ($P = 0.157$). Furthermore, the Schirmer test results did not improve markedly (10.36 ± 6.89 and 12.43 ± 9.43 mm/5 min, respectively; $P = 0.132$).

Discussion

Despite being a highly prevalent condition, the clinical diagnosis and management of DED remain problematic. As MMP-9 has been implicated to have a crucial role in inflammation in DED, we assessed the usefulness of using graded InflammaDry assay results for predicting response to cyclosporine treatment in DED. We found that the semiquantitative InflammaDry results correlated with DED symptoms; this approach has value in determining patient status and predicting their treatment response.

DED is a prevalent and chronic condition that affects quality of life[20] and has a psychologic impact on the affected individuals.[21] There is a discrepancy in disease recognition between patients and ophthalmologists, which may be associated with the difficulties in proper diagnosis and treatment of DED. Therefore, the use of a point-of-care MMP-9 immunoassay (InflammaDry) has been investigated as a supplementary tool for DED diagnosis. However, to date, a wide range of MMP-9 positivity has been reported using this assay in DED patients. Sambursky et al.[15] reported the sensitivity and specificity of InflammaDry as 85% and 94% in their prospective, multicenter clinical trial incorporating 143 DED patients and 63 healthy individuals. In their next study, they reported positive and negative agreements of InflammaDry as 81% and 98%, respectively, in a study of 237 DED patients.[16] However, more recent studies have shown disappointing outcomes. Schargus et al.[22] reported that 11% of the symptomatic and 14% of the suspected mild DED patients had positive InflammaDry test results. Lanza et al.[23] reported 39% positivity for InflammaDry in DED patients. Moreover, MMP-9 positivity was 40.4% in DED patients and 5.6% in healthy controls as reported by Messmer et al.[17] In our study, the positivity was relatively high, with 77.5% in DED patients and 7.5% in healthy subjects. Different diagnostic criteria for DED were used in each study, which may underlie the considerable variation in reported positivity.

However, there is substantial evidence that positive MMP-9 results correlate well with signs and symptoms of DED. Aragona et al.[8] quantified the levels of MMP-9 with real-time polymerase chain reaction (RT-PCR), and found that MMP-9 levels correlated well with TBUT, conjunctival stain, and corneal stain results. Moreover, Messmer et al.[17] proved that positive InflammaDry results correlated well with OSDI scores, TBUT, Schirmer test results, conjunc-

Table 5. Topical Cyclosporine Treatment Response in MMP-9–Negative DED Patients

Characteristics	Before Treatment	After Treatment	P Value
MMP-9 grade (positive/negative, trace/weak/positive/strong)	0/14 (0/0/0/0)	6/8 (1/5/1/1)	0.010
OSDI	36.72 ± 36.02	37.46 ± 34.20	0.561
TBUT (seconds)	5.79 ± 2.86	6.57 ± 2.17	0.209
Corneal staining (score 0/1/2)	0.29 ± 0.61 (11/2/1)	0.14 ± 0.36 (12/2/0)	0.157
Schirmer test (mm/5 min)	10.36 ± 6.89	12.43 ± 9.43	0.132

translational vision science & technology

tival staining, corneal staining, as well as the number of obstructed Meibomian ducts and pathologic Meibomian gland secretion.

RT-PCR and the InflammaDry test have drawbacks. Clinical application of RT-PCR is somewhat complicated, more expensive, and takes a longer time to obtain results. In contrast, correlating signs and symptoms of DED with positivity of InflammaDry may not be clinically meaningful, as the InflammaDry result is dichotomous, corresponding to either one of "negative" or "positive." Therefore, we introduced a semiquantitative method of grading the intensity of the InflammaDry test, and assessed the correlation of the grading index with the diverse symptoms and signs of DED. We found that increasing MMP-9 levels correlated well with OSDI scores, TBUT, corneal staining, and Schirmer test results. Additionally, InflammaDry may not only correlate with the symptoms and signs of DED patients, but also may be used as a tool for monitoring the outcomes of DED treatment.

Nevertheless, this semiquantitative method of InflammaDry grading has the drawback in terms of the occasional ambiguity of comparing the InflammaDry result with the grading index, which resulted in discrepant interpretations between observers. Direct visualization and comparison of the intensity of the InflammaDry result may not be completely objective, and masking the observers from clinical information was absolutely necessary. However, the agreement among the three observers was considerably higher than expected. Although the reproducibility of this method still requires further study, it may serve as a reliable tool.

The foremost finding of this study was the predictive value of the InflammaDry assay results in terms of the response to topical cyclosporine treatment. One previous study suggested that MMP-9 may have predictive value for anti-inflammatory treatment in DED.[24] In our study, MMP-9–positive patients showed a more favorable response to topical cyclosporine than MMP-9–negative patients, with decreased MMP-9 levels, improved OSDI scores, and increased TBUT, and Schirmer test results after a month of treatment. Excluding participants with a previous history of topical anti-inflammatory treatment may have contributions to this result.

MMP-9 activity is regulated by several mechanisms, including epigenetic processes, cell–cell interactions, and cytokine-mediated pathways.[24] The hyperosmolarity of the tear film triggers the SAPK signaling cascade, which in turn releases MMP-9 from corneal epithelial cells.[13] Additionally, cytokines derived from mitogen-activated protein kinases and NF-kβ activate neutrophils to secrete MMP-9 in its proenzyme form,[25] which is later activated by other proteinases.[26] Among its various activities, MMP-9 is the most efficient activator of precursor IL-1β.[27,28] IL-1β modulates adaptive immune responses involving T- and B-cells.[29] It is not surprising that topical cyclosporine, which inhibits T-cell activation,[30] may work more efficiently in DED patients with high MMP-9 levels. Topical cyclosporine already is known to be more efficacious in Sjögren's disease, which involves a cell-mediated pathway and, therefore, a predominantly adaptive mechanism, than in evaporative dry eye, which is mainly based on an innate immune response.[31,32] This is consistent with the study performed by Aragona et al.,[8] which reported significantly higher expression of MMP-9 in Sjögren's syndrome patients than in Meibomian gland dysfunction patients. Higher MMP-9 levels in the tear film of Sjögren's syndrome patients may be associated with their more favorable response to topical cyclosporine treatment.

The outcomes of this study indicated that the results of the InflammaDry test may serve as a treatment criterion for initiating topical cyclosporine treatment of DED patients. Although topical cyclosporine is relatively safe, some reported adverse effects, including ocular burning and stinging sensations, may occur.[33] When a drug is prescribed, whether the advantages outweigh the disadvantages always should be considered, and topical cyclosporine must be used with caution. The favorable treatment outcomes in MMP-9–positive patients suggested that the InflammaDry may function as a reference point for initiating topical cyclosporine treatment.

Our study has some limitations. First, due to a relatively small population size, we had to enroll both of the patients' eyes, which might overcompensate the results of the statistics. Further studies with larger sample size would be required to draw the results more clearly. Second, regarding the recruitment method of our study, some form of selection bias may have occurred. An adequately powered case–control study with age-, sex-, and ethnicity-matched controls would have reduced the bias.

In conclusion, the semiquantitative grading of InflammaDry results correlated well with the symptoms and signs of DED, and may be used to predict patient status and monitor treatment response. In addition, MMP-9–positive patients showed more favorable responses to topical cyclosporine than

MMP-9–negative patients, suggesting that the InflammaDry result may function as a reference point for commencing topical cyclosporine treatment. Further studies regarding the reproducibility of the test and monitoring of response to other drugs, such as topical corticosteroid, may further improve our understanding of the role of MMP-9 in this condition, and the conceivable applications of this information.

Acknowledgments

References

1. The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye WorkShop (2007). *Ocul Surf*. 2007;5:75–92.
2. The epidemiology of dry eye disease: report of the Epidemiology Subcommittee of the International Dry Eye WorkShop (2007). *Ocul Surf*. 2007;5:93–107.
3. Pflugfelder SC, Tseng SC, Sanabria O, et al. Evaluation of subjective assessments and objective diagnostic tests for diagnosing tear-film disorders known to cause ocular irritation. *Cornea*. 1998;17:38–56.
4. Luo L, Li DQ, Corrales RM, Pflugfelder SC. Hyperosmolar saline is a proinflammatory stress on the mouse ocular surface. *Eye Contact Lens*. 2005;31(5):186–193.
5. Luo L, Li DQ, Doshi A, Farley W, Corrales RM, Pflugfelder SC. Experimental dry eye stimulates production of inflammatory cytokines and MMP-9 and activates MAPK signaling pathways on the ocular surface. *Invest Ophthalmol Vis Sci*. 2004;45:4293–4301.
6. De Paiva CS, Villarreal AL, Corrales RM, et al. Dry eye-induced conjunctival epithelial squamous metaplasia is modulated by interferon-gamma. *Invest Ophthalmol Vis Sci*. 2007;48:2553–2560.
7. Chi W, Hua X, Chen X, et al. Mitochondrial DNA oxidation induces imbalanced activity of NLRP3/NLRP6 inflammasomes by activation of caspase-8 and BRCC36 in dry eye. *J Autoimmun*. 2017;80:65–76.
8. Aragona P, Aguennouz M, Rania L, et al. Matrix metalloproteinase 9 and transglutaminase 2 expression at the ocular surface in patients with different forms of dry eye disease. *Ophthalmology*. 2015;122:62–71.
9. Chotikavanich S, de Paiva CS, Li de Q, et al. Production and activity of matrix metalloproteinase-9 on the ocular surface increase in dysfunctional tear syndrome. *Invest Ophthalmol Vis Sci*. 2009;50:3203–3209.
10. De Paiva CS, Corrales RM, Villarreal AL, et al. Corticosteroid and doxycycline suppress MMP-9 and inflammatory cytokine expression, MAPK activation in the corneal epithelium in experimental dry eye. *Exp Eye Res*. 2006;83:526–535.
11. Stern ME, Pflugfelder SC. Inflammation in dry eye. *Ocul Surf*. 2004;2:124–130.
12. Tseng HC, Lee IT, Lin CC, et al. IL-1beta promotes corneal epithelial cell migration by increasing MMP-9 expression through NF-kappaB- and AP-1-dependent pathways. *PLoS One*. 2013;8:e57955.
13. Hessen M, Akpek EK. Dry eye: an inflammatory ocular disease. *J Ophthalmic Vis Res*. 2014;9:240–250.
14. Corrales RM, Stern ME, De Paiva CS, Welch J, Li DQ, Pflugfelder SC. Desiccating stress stimulates expression of matrix metalloproteinases by the corneal epithelium. *Invest Ophthalmol Vis Sci*. 2006;47:3293–3302.
15. Sambursky R, Davitt WF, 3rd, Latkany R, et al. Sensitivity and specificity of a point-of-care matrix metalloproteinase 9 immunoassay for diagnosing inflammation related to dry eye. *JAMA Ophthalmol*. 2013;131:24–28.
16. Sambursky R, Davitt WF III, Friedberg M, Tauber S. Prospective, multicenter, clinical evaluation of point-of-care matrix metalloproteinase-9 test for confirming dry eye disease. *Cornea*. 2014;33:812–818.
17. Messmer EM, von Lindenfels V, Garbe A, Kampik A. Matrix metalloproteinase 9 testing in dry eye disease using a commercially available point-of-care immunoassay. *Ophthalmology*. 2016;123:2300–2308.
18. Whitcher JP, Shiboski CH, Shiboski SC, et al; Sjögren's International Collaborative Clinical Alliance Research Groups. A simplified quantitative method for assessing keratoconjunctivitis sicca from the Sjogren's Syndrome International Registry. *Am J Ophthalmol*. 2010;149:405–415.

19. Sacchetti M, Mantelli F, Lambiase A, Mastropasqua A, Merlo D, Bonini S. Systematic review of randomised clinical trials on topical ciclosporin A for the treatment of dry eye disease. *Br J Ophthalmol*. 2014;98:1016–1022.

20. Vitale S, Goodman LA, Reed GF, Smith JA. Comparison of the NEI-VFQ and OSDI questionnaires in patients with Sjogren's syndrome-related dry eye. *Health Qual Life Outcomes*. 2004; 2:44.

21. Schiffman RM, Walt JG, Jacobsen G, Doyle JJ, Lebovics G, Sumner W. Utility assessment among patients with dry eye disease. *Ophthalmology*. 2003;110:1412–1419.

22. Schargus M, Ivanova S, Kakkassery V, Dick HB, Joachim S. Correlation of tear film osmolarity and 2 different MMP-9 tests with common dry eye tests in a cohort of non-dry eye patients. *Cornea*. 2015;34:739–744.

23. Lanza NL, McClellan AL, Batawi H, et al. Dry eye profiles in patients with a positive elevated surface matrix metalloproteinase 9 point-of-care test versus negative patients. *Ocul Surf*. 2016;14: 216–223.

24. Lanza NL, Valenzuela F, Perez VL, Galor A. The matrix metalloproteinase 9 point-of-care test in dry eye. *Ocul Surf*. 2016;14:189–195.

25. Lin CC, Kuo CT, Cheng CY, et al. IL-1 beta promotes A549 cell migration via MAPKs/AP-1- and NF-kappaB-dependent matrix metalloproteinase-9 expression. *Cell Signal*. 2009;21:1652–1662.

26. Klein T, Bischoff R. Physiology and pathophysiology of matrix metalloproteases. *Amino Acids*. 2011;41:271–290.

27. Solomon A, Dursun D, Liu Z, Xie Y, Macri A, Pflugfelder SC. Pro- and anti-inflammatory forms of interleukin-1 in the tear fluid and conjunctiva of patients with dry-eye disease. *Invest Ophthalmol Vis Sci*. 2001;42:2283–2292.

28. Schonbeck U, Mach F, Libby P. Generation of biologically active IL-1 beta by matrix metalloproteinases: a novel caspase-1-independent pathway of IL-1 beta processing. *J Immunol* 1998;161:3340–3346.

29. Rao DA, Tracey KJ, Pober JS. IL-1alpha and IL-1beta are endogenous mediators linking cell injury to the adaptive alloimmune response. *J Immunol*. 2007;179:6536–6546.

30. Fruman DA, Klee CB, Bierer BE, Burakoff SJ. Calcineurin phosphatase activity in T lymphocytes is inhibited by FK 506 and cyclosporin A. *Proc Natl Acad Sci U S A*. 1992;89:3686–3690.

31. Musumeci M, Aragona P, Bellin M, et al. Acidic mammalian chitinase in dry eye conditions. *Cornea*. 2009;28:667–672.

32. Zhang X, Schaumburg CS, Coursey TG, et al. CD8(+) cells regulate the T helper-17 response in an experimental murine model of Sjogren syndrome. *Mucosal Immunol*. 2014;7:417–427.

33. Barber LD, Pflugfelder SC, Tauber J, Foulks GN. Phase III safety evaluation of cyclosporine 0.1% ophthalmic emulsion administered twice daily to dry eye disease patients for up to 3 years. *Ophthalmology*. 2005;112:1790–1794.