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Production and Activity of Matrix Metalloproteinase-9 on the Ocular Surface Increase in Dysfunctional Tear Syndrome

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Abstract

Purpose—To evaluate production and activity of metalloproteinase (MMP)-9 on the ocular surface of patients with dysfunctional tear syndrome (DTS) and determine any correlation between MMP-9 activity and clinical parameters.

Methods—Forty-six patients with newly diagnosed DTS and 18 control subjects were recruited. Complete ocular surface examinations were performed. Tear MMP-9 activity was assessed with an MMP-9 activity assay in 1 μ L of unstimulated tear fluid. Using conjunctival epithelial cells from 19 patients with DTS and 16 controls, levels of MMP-9 and its regulating cytokine mRNA transcripts were evaluated by semiquantitative real-time PCR.

Results—Each of four DTS severity-based groups had significantly higher mean MMP-9 activities than did the control group, which was 8.39 ± 4.70 ng/mL. The DTS4 group had the highest MMP-9 activity (381.24 ± 142.83 ng/mL), for which the mean was significantly higher than that of other DTS groups. In addition, patients with DTS had significantly higher levels of IL-1 β , IL-6, TNF- α , and TGF- β 1 mRNA transcripts in their conjunctival epithelia than did the control subjects. Tear MMP-9 activities showed significant correlation with symptom severity scores, decreased low-contrast visual acuity, fluorescein tear break-up time, corneal and conjunctival fluorescein staining, topographic surface regularity index (SRI), and percentage area of abnormal superficial corneal epithelia by confocal microscopy.

Conclusions—Tear MMP-9 activity was significantly higher in patients with DTS. This activity was associated with increased mRNA expression of MMP-9 and its regulating genes and correlated strongly with clinical parameters. MMP-9 appears to be a potentially useful biomarker for diagnosing, classifying, and monitoring DTS.

It has been proposed that inflammatory mechanisms are involved in the pathophysiology of dysfunctional tear syndrome (DTS), the more encompassing term for dry eye disease, proposed by the Delphi Dry Eye Panel Report in 2006.¹ It is now recognized that changes in tear composition in DTS may destabilize the tear film and cause ocular surface epithelial disease.^{1,2–6} Matrix metalloproteinases (MMPs) are proteolytic enzymes produced by

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stressed ocular surface and glandular epithelial cells, as well as by the inflammatory/immune cells that infiltrate these tissues. Increased activity of MMPs has been implicated in these pathologic ocular surface changes.^{5,7}

MMPs play a vital role in wound healing and inflammation.^{8,9} Increased levels of MMP-3 and -9 have been detected in the tear fluid of patients with keratoconjunctivitis sicca (KCS).^{10,11} Among the MMPs, MMP-9 has been found to be of central importance in cleaving epithelial basement membrane components and tight junction proteins (such as ZO-1 and occludin) that maintain corneal epithelial barrier function.^{12–14} MMP-9 belongs to the gelatinase group of metalloproteinases that degrade denatured collagen; native collagens type IV, V, and VII; and elastin. Expression of MMP-9 by the ocular surface epithelia in normal healthy eyes is low.¹⁵ Increased production of MMP-9 by the corneal epithelium has been found in the eyes of individuals with sterile corneal ulceration.¹⁶ Increased MMP-9 activity has been associated with disruption of corneal epithelial barrier function and corneal surface irregularity in an experimental murine model of dry eye.¹⁷ MMP-9 knockout mice showed significantly less alteration of epithelial barrier function in response to experimental desiccating stress than did wild-type mice.¹⁷ This protective effect was abrogated by topical application of MMP-9 to the ocular surface.¹⁷

Previous human studies have found an increased concentration of pro-MMP-9 measured by enzyme-linked immunosorbent assay (ELISA) in tear fluid of patients with ocular rosacea.^{5,10} Solomon et al.⁴ found increased activity of MMP-9 in the tear fluid of patients with meibomian gland disease and Sjögren's syndrome.⁴ Most MMPs are secreted as inactive zymogens (proMMPs) that require extracellular activation before they are able to cleave extracellular matrix components. This study was designed to measure the activity of the total active form of MMP-9 in normal eyes and in those in the various DTS groups classified by the Delphi panel and the Dry Eye Workshop (DEWS). The correlation between tear MMP-9 activity and clinical parameters of DTS was determined. Finally, mRNA transcript levels of MMP-9 and its regulating cytokines in the conjunctival epithelium were measured.

Methods

The clinical protocol was approved by the Baylor College of Medicine Institutional Review Board and was in accordance with the tenets of the Declaration of Helsinki. After providing informed consent, 46 patients with DTS, newly diagnosed by a single investigator (SCP), and 18 asymptomatic control subjects were recruited for measurement of tear MMP-9 activity. A subset of 19 patients with newly diagnosed DTS and 16 asymptomatic control subjects were also enrolled for evaluating gene expression in the conjunctival epithelium. The following ocular surface and tear examinations were sequentially performed on all study participants: completion of the Ocular Surface Disease Index (OSDI) symptom questionnaire,^{18,19} collection of unstimulated tear samples, fluorescein tear break-up time (TBUT), corneal and conjunctival fluorescein staining, and Schirmer I test. Finally, impression cytology to obtain cells for evaluating gene expression was performed.

Criteria for diagnosis of DTS included an OSDI score >20 ^{1,18–20} with one or more of the following signs: TBUT <7 seconds,^{19,21} punctate corneal fluorescein staining, or Schirmer I score <10 mm. Subjects were excluded if they were using any topical medications or if they had another ocular surface disease.

The patients were classified into four levels of severity according to reported criteria of the DEWS²² report, as shown in Table 1. Lid margin disease and tear signs could be present at any severity level. When not all criteria of a severity group were met, severity grading was

based on the worst parameter. Asymptomatic control subjects were recruited from employees of Baylor College of Medicine presenting for routine eye examinations.

Participants completed the OSDI questionnaire containing 12 items measuring visual function, ocular irritation symptoms, and effects of stressful environmental conditions.¹⁸ The score ranged from 12 (no symptoms) to a maximum of 64.

Measurement of High- and 10% Low-Contrast Visual Acuity

High (100%)- and low (10%)-contrast best corrected visual acuity was measured with a standard EDTRS chart mounted on a stand 4 m from the spectacle plane of the subject. The background luminance of the chart was set at 85 cd/m², and ambient luminance was adjusted to 3 cd/m².^{23,24} The difference between high- and low-contrast acuity was recorded.

Computerized Videokeratography

Videokeratographic examination (TMS-2 Corneal Topography System; Tomey, Waltham, MA) was performed as previously reported.²⁰ The SRI was calculated by the instrument's software and recorded.

Tear Fluid Collection

Tear fluid (0.5 μ L) was atraumatically collected with a 0.5- μ L glass capillary tube (Drummond Scientific, Broomall, PA) by capillary action from the inferior tear meniscus of each eye. Tear samples from both eyes (1 μ L total) were eluted into a sterile tube containing 9 μ L of PBS and 0.1% bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, MO). The tubes were sealed with a cap containing a rubber O-ring to prevent evaporation and immediately stored at -80°C until activity assays were performed.

Fluorescein TBUT

After the inferior tarsal conjunctiva was touched with a sodium fluorescein strip (Fluor-I-Strip; Bausch & Lomb Pharmaceuticals Inc., Tampa, FL) wet with preservative-free saline (Unisol; Alcon, Fort Worth, TX), the precorneal tear film was examined under blue-light illumination, as previously reported.²⁰

Conjunctival and Corneal Fluorescein Staining

The ocular surface was examined 2 minutes after fluorescein instillation into the tear film, as described earlier. The intensity of conjunctival fluorescein staining was recorded with a modified van Bijsterveld grading scheme on a scale of 0 (none) to 3 (confluent) in the nasal conjunctiva and temporal conjunctiva.²⁵ The Baylor grading scheme was used to grade the intensity of corneal fluorescein staining²⁶ 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 dots, 0; 1 to 5 dots, 1; 6 to 15 dots, 2; 16 to 30 dots, 3; >30 dots, 4. If there is one area of confluence add 1; two or more areas of confluence, add 2; filamentary keratitis, add 2.

Schirmer I Test

A Schirmer I test was performed by placing Schirmer strips (Alcon) over the lower lid margin, at the junction of the lateral and middle thirds, for 5 minutes. The length of strip wetting was recorded in millimeters.

Confocal Microscopy

Confocal microscopy was performed on the cornea of a subset of seven patients with DTS and three normal control subjects (Retina Tomograph II in combination with the Rostock

Cornea Module; Heidelberg Engineering, Heidelberg, Germany). After topical application of anesthesia, a drop of optical coupling medium gel (GenTeal, 0.3% hypromellose; Novartis Ophthalmics, Basel, Switzerland) was applied to the inferior conjunctival fornix. Images were sequentially captured from the air superficial corneal epithelial interface posterior to the subepithelial nerve plexus. For analysis, the area of abnormal superficial epithelia, defined by singular or multiple hyperreflective opaque cells or cells with pyknotic or snake like nuclei was measured in digital images with NIHJ software (developed by Wayne Rasband, National Institutes of Health, Bethesda, MD; available at <http://rsb.info.nih.gov/ij/index.html>) by two masked observers. The abnormal area was calculated as the average value of the percentage over the total ($400 \times 400 \mu\text{m}$) field area of four randomly selected areas in the central cornea.

Collection of Conjunctival Epithelial cells

Conjunctival epithelial cells were obtained by an impression cytology technique. One drop of anesthetic was instilled into both eyes, and excess fluid was dried with a tissue. Sterile nitrocellulose filter papers ($0.45 \mu\text{m}$ HA, 45 mm circles, Cat No. HAWP04700; Millipore, Bedford, MA) with a tapered rectangular cut of $5 \times 8 \times 6 \times 10 \text{ mm}$ were applied to the temporal and inferior bulbar conjunctiva of each eye. The papers were gently peeled off the surface of the conjunctiva with a pair of forceps and placed in a tube containing RNA lysis buffer.

Tear MMP-9 Activity

Total MMP-9 enzyme activity was measured with an MMP activity assay kit (Biotrak; Amersham Biosciences, Piscataway, NJ) according to the manufacturer's protocol. In brief, $100 \mu\text{L}$ of each pro-MMP standard ($0.125\text{--}4 \text{ ng/mL}$), diluted tears ($1 \mu\text{L}$ of tears in $9 \mu\text{L}$ PBS and 0.1% BSA diluted in $90 \mu\text{L}$ assay buffer), and assay buffer (for blanks) were incubated at 4°C overnight in wells of a microtiter plate precoated with anti-MMP-9 mouse monoclonal capture antibody. Plates were washed four times with 0.01 M sodium phosphate buffer (pH 7.0) containing 0.05% Tween-20. Total MMP-9 activity was measured by activating bound pro-MMP-9 with $50 \mu\text{L}$ of 1 mM *p*-amino phenylmercuric acetate (APMA) in assay buffer at 37°C for 1.5 hours. Detection reagent ($50 \mu\text{L}$) was added to each well, and samples were incubated at 37°C for six hours. Active MMP-9 was detected through its ability to activate a modified prodetection enzyme that subsequently cleaved its chromogenic peptide substrate. Absorbance was read at 405 nm in a microplate reader (Versamax; Molecular Devices, Sunnyvale, CA). The activity of MMP-9 in a sample was determined by interpolation from a standard curve. Tear sample absorbance readings were multiplied by a dilution factor of 100.

RNA Isolation and Real-Time PCR

Gene expression in the conjunctival epithelium was evaluated in a subset of 19 DTS and 16 normal subjects. Conjunctival epithelial RNA, enriched for mRNA, was isolated from impression cytology samples by using guanidine-isothiocyanate-containing lysis buffer followed by selective binding of RNA to the silica-gel-based membrane (RNeasy Micro kit; Qiagen, Gaithersburg, MD). The RNA concentration was measured by its absorption at 260 nm, and the samples were stored at -80°C until used for polymerase chain reaction (PCR). First-strand cDNA was synthesized from $0.2 \mu\text{g}$ of total RNA with random hexamers by M-MuLV reverse transcription (Ready-To-Go You-Prime First-Strand Beads; GE Health Care, Inc., Arlington Heights, IL). Real-time PCR was performed with specific probes (TaqMan MGB; Applied Biosystems, Inc. [ABI], Foster City, CA) for GAPDH, MMP-9, MMP-3, IL-1 β , IL-6, TNF- α , and TGF- β 1 (Assay IDs: Hs 99999905_m1, Hs 00234579_m1, Hs 00233962_m1, Hs 00174097_m1, Hs 00174131_m1, Hs 00174128_m1, and Hs 99999918_m1, respectively), with PCR master mix (TaqMan Gene Expression Master Mix;

ABI), in a commercial thermocycling system (Mx3005P QPCR System; Stratagene, La Jolla, CA), according to the manufacturer's recommendations. Assays were performed in duplicate in each experiment. The results of quantitative PCR were analyzed by the comparative CT method.²⁷ where the target change was $2^{-\Delta\Delta C_t}$. The cycle threshold (C_t) was determined using the primary (fluorescent) signal as the cycle at which the signal crosses a user-defined threshold. The results were normalized by the C_t value of GAPDH, and the relative mRNA level in the normal control group was used as the calibrator.

Statistical Analysis

Statistical analyses were performed with commercial software (Prism; GraphPad, La Jolla, CA, and Excel; Microsoft, Redmond, WA). Data are expressed as the mean $\pm$ SD. The normality of data was checked with the Kolmogorov-Smirnov test using the Dallal and Wilkinson approximation. One-way analysis of variance (ANOVA) or the Kruskal-Wallis test was used to detect statistical differences among multiple groups with normal or non-normal distribution, respectively. Statistical comparisons of tear MMP-9 activity levels between groups were performed by two-sample *t*-test. Inflammatory cytokine mRNA transcript levels were compared with the unpaired two-tailed *t*-test. Correlations between tear MMP-9 activities and clinical parameters, including symptom severity scores, decrease of low-contrast visual acuity, TBUT, and percentage area of abnormal superficial corneal epithelia by confocal microscopy were determined by logarithmic regression. Correlations between tear MMP-9 activities and SRI scores, corneal fluorescein staining scores, and conjunctival fluorescein staining scores were determined by polynomial regression. Statistical significance was calculated by Spearman correlation, which makes no assumption about the normality of the data. $P < 0.05$ was considered to be statistically significant.

Results

Features of Study Groups

Forty-six patients with DTS with a mean age of 54.7 ± 14.7 (80% female, 20% male) and 18 controls with a mean age of 41.7 ± 13.0 (72% female, 28% male) were included for tear MMP-9 activity measurement.

The clinical features of control subjects and DTS subjects stratified by severity level are provided in Table 2. Associated ocular surface diseases found in each DTS severity level are noted in Table 3.

Clinical Parameters

Patients with DTS had a significantly greater mean decrease in 10% low-contrast visual acuity (0.24 ± 0.06 logMAR) than did control subjects (0.17 ± 0.04 logMAR) ($P = 0.001$). The decrease in low-contrast acuity showed significant and positive correlation with responses to two questions about blurred symptoms on the OSDI questionnaire ($r^2 = 0.39$, $P < 0.001$), but this correlation was not found for high-contrast acuity ($r^2 = 0.07$, $P = 0.16$).

In the subset of subjects evaluated by confocal microscopy, the percentage area of abnormal superficial corneal epithelial cells measured over the total image field area was $17.35\% \pm 15.41\%$ in subjects with DTS (DTS2 [$n = 3$], DTS3 [$n = 3$], and DTS4 [$n = 1$]), compared with $0.34\% \pm 0.38\%$ in control subjects ($n = 3$; $P < 0.03$). Some eyes with DTS had abnormal superficial corneal epithelia with a polygonal shape and highly reflective cytoplasm, with bright-appearing (pyknotic) nuclei surrounded by a dark perinuclear space. Representative confocal images are shown in Figure 1.

Tear MMP-9 Activity

The tear MMP-9 activities in all groups of patients with DTS are presented in Table 4. There was no significant difference in tear MMP-9 activity between male and female participants in the normal and DTS groups. Although the mean age of patients with DTS was higher than that of the normal control subject (54.7 vs. 41.7 years, $P=0.01$), there was no significant difference in tear MMP-9 activity in each decade of age between 20 and 80 years in the normal and DTS groups.

Expression of MMP-9 and Regulating Cytokine Genes

Levels of mRNA transcripts encoding MMP-9 and its regulating inflammatory cytokines in conjunctival epithelia obtained by impression cytology from 19 patients with DTS, recruited from DTS1 ($n=3$), DTS2 ($n=2$), DTS3 ($n=4$), and DTS4 ($n=10$) and 16 normal subjects, evaluated by real-time PCR, are shown in Figure 2. Significantly higher levels of MMP-9, IL-1 β , IL-6, TNF- α , and TGF- β 1 transcripts were observed in patients with DTS than in normal subjects ($P<0.05$). MMP-3 transcripts were undetectable in samples obtained from normal subjects and those with DTS.

Correlation of Tear MMP-9 Activities with Clinical Parameters

The correlations between tear MMP-9 activity and all clinical parameters were evaluated (Fig. 3). Tear MMP activities showed significant and positive correlation with symptom severity scores, SRI scores, corneal fluorescein staining scores, conjunctival fluorescein staining scores ($P<0.001$), decreased low-contrast visual acuity ($P=0.002$), and showed significant and negative correlation with fluorescein TBUT ($P<0.001$). They also showed significant and positive correlation with percentage area of abnormal superficial corneal epithelia in confocal images ($r^2=0.64$, adjusted $r^2=0.57$, $P=0.005$) in the subset of patients subjected to confocal microscopy.

Discussion

DTS is a common ocular surface disease that can affect productivity and quality of life. Diagnostic criteria and management guidelines for DTS have been proposed by the Delphi panel and DEWS workshop. In this study, tear MMP-9 activity was evaluated in groups of patients with DTS classified by severity.

Each of the DTS groups had significantly higher mean levels of tear MMP-9 activity than the normal subjects. Moreover, the most severe DTS group (DTS4) was found to have the highest mean MMP-9 activity, significantly higher than the other DTS groups. The mean MMP-9 activities in the DTS1 and -2 groups were not significantly different from each other; however, the DTS3 group had significantly higher mean MMP-9 activity than the DTS1 group. These findings indicate that tear MMP-9 activity is significantly elevated, even in mild DTS and that this may be a more sensitive diagnostic marker than clinical signs. This elevation appears to be clinically significant, and tear MMP-9 activity may prove to be a better marker of disease severity than traditional clinical signs.

Tear MMP-9 activity showed strong correlation with the results of conventional diagnostic tests of DTS (symptom severity scores, SRI scores, fluorescein TBUT, and corneal and conjunctival fluorescein staining scores). Those clinical findings certainly can be attributed to the reported ability of MMP-9 to degrade epithelial tight junction and basement membrane proteins, leading to altered epithelial permeability and poor epithelial adherence.¹⁷

In the present study, we also measured 10% low-contrast visual acuity. Although the impact of DTS on visual function can be assessed by 100% high-contrast visual acuity, low-contrast vision may be a more functional and sensitive measure of the impact of this condition on visual quality. Previous studies in normal subjects have found that mesopic low-contrast acuity shows a stronger correlation with retinal image quality than does mesopic or photopic high-contrast acuity.^{28,29} Moreover, this technique enabled detection of loss of vision in keratoconus that was not detected with high-contrast testing.³⁰ In addition, a previous study found that there was greater impact on restoration of low-contrast visual acuity when performing a Zernike-based optical correction.³¹ In our study, besides finding a significant correlation between the decrease of 10% low-contrast visual acuity and tear MMP-9 activities, we also found that the decrease in low-contrast acuity correlated more significantly with responses to the two questions about blurred symptoms on the OSDI questionnaire than did 100% high-contrast acuity.

Because of its ability to reveal clinicomorphologic correlations at the cellular level, the confocal microscope is becoming an increasingly valuable clinical tool. The normal superficial corneal epithelial cell was previously reported to have a dark cell nucleus and cytoplasm with a bright border.³² Previous confocal microscopic studies of dry eyes found a decreased density of corneal nerves and superficial corneal epithelia, but not of basal cells.^{33,34} In the present study, a correlation was found between tear MMP-9 activity and the percentage area of those abnormal superficial corneal epithelial cells. This change in the optical characteristics of the superficial epithelia may signal an early stage of epithelial desquamation induced by desiccating stress and increased tear protease activity.

Stimulated production of MMP-9, as well as cytokines that stimulate MMP-9 production (IL-1, IL-6, TNF- α , and TGF- β 1) by the ocular surface epithelia was confirmed at the transcriptional level by semiquantitative real-time PCR in our study. Similar results were also observed by Aragona et al. (*IOVS* 2008;49:ARVO E-Abstract 123). The increased tear film osmolarity that accompanies DTS is recognized as a proinflammatory stimulus.³⁵ Exposure of cultured human corneal epithelial cells to medium of increasing osmolarity has been found to stimulate production of MMP-9 and several inflammatory cytokines, such as IL-1 β and TNF- α (Li D, et al. *IOVS* 2002;43:ARVO E-Abstract 1981; Luo L, et al. *IOVS* 2003;44:ARVO E-Abstract 1026). Those inflammatory cytokines, in turn, have been shown to stimulate the production of a variety of MMPs, including MMP-9 by corneal epithelium.^{36,37}

Among its various activities, MMP-9 is known to activate precursor IL-1 β and latent TGF- β 1 into their active forms. Among several proteases, MMP-9 was found to be the most efficient activator of precursor IL-1 β .^{4,38} Therefore, the increase of MMP-9 activity on the ocular surface can amplify the chronic immune-based inflammation in DTS. Indeed, MMP-9 has been demonstrated in a previous study to speed cornea epithelial regeneration by modulating the inflammatory response in the healing cornea.³⁹ These findings indicate that DTS is capable of initiating an escalating cycle of cytokines and proteinases that can have deleterious consequences for the ocular surface.

Our finding of highly increased IL-6 gene expression in the conjunctival epithelium is consistent with several previously reported studies in both Sjögren- and non-Sjögren-associated aqueous tear deficiency.^{3,40,41} Significantly elevated IL-6 concentrations in tear fluid of patients with dry eye have been found in association with increased epithelial expression of IL-6.^{42,43} It should be noted that it was reported in another study that out of a spectrum of ocular surface diseases, including dry eye, IL-6 was found to be elevated only in eyes with conjunctivochalasis.⁴⁴ Of interest, this study found that the concentration of MMP-9 was elevated in all the ocular surface conditions compared with concentrations in

control eyes. This finding supports the concept that elevation of tear MMP-9 is a common feature of ocular surface epithelial diseases, regardless of cause¹⁵ and is consistent with our finding that the mean MMP-9 activity in tears was increased in the variety of conditions associated with DTS noted in Table 3 and that tear MMP-9 activity showed strong correlation with the severity of corneal and conjunctival epithelial disease.

MMP-9 is secreted as a latent proenzyme that requires extracellular activation to be functional. In vitro, MMPs can be activated by chemical and physical agents, such as aminophenylmercuric acetate (AMPA), low pH, and heat. In vivo, MMPs are generally activated by other proteinases. MMP-9 has been reported to be activated by plasmin and more efficiently by MMP-3.^{10,45,46} A previous study by our group found an increase in MMP-3 mRNA transcripts in cultured human corneal epithelial cells treated with IL-17 by real-time PCR using 1 μ g of RNA.⁴⁷ Using the same method, we were unable to detect MMP-3 transcripts in the conjunctival epithelium in our present study; however, a lower amount of total RNA was obtained by impression cytology which may have decreased sensitivity. Indeed, in a previous study, detection of MMP-3 transcripts by real-time PCR with 2 μ g of RNA obtained from retinoic-acid-treated cultured human conjunctival epithelial cells was reported.⁴⁸ Other possible explanations for not detecting MMP-3 are that it is produced by stromal cells or inflammatory cells that are in low density in the cytology samples, or MMP-3 production is lower in the conjunctival than in the corneal epithelium.^{49–52} For the tear MMP-9 activity assay, we initially attempted to measure levels of the active MMP-9 enzyme in tear fluid without AMPA treatment; however, the levels were found to be too low to permit statistical comparison.

In summary, we found increased mean tear MMP-9 activity in all groups of patients with DTS, which was confirmed at the gene transcriptional level. In the DTS3 and -4 groups, the mean tear MMP-9 activity was found to be significantly higher than that in the other groups of patients with DTS. The MMP-9 activities were also found to correlate strongly with clinical parameters. This minimally invasive and sensitive MMP-9 assay is capable of evaluating the vital role of MMP-9 and may be clinically helpful for diagnosing, classifying, and monitoring DTS. It may prove to be an important clinical parameter in a future dry eye clinical trial.

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Figure 1. Representative confocal images of superficial corneal epithelium of (A) normal control, (B) DTS2, (C) DTS3, and (D) DTS4 subjects. Scale bar, 50 μm .

Figure 2.

Relative levels of MMP-9, IL-1 β , IL-6, TNF- α , and TGF- β 1 mRNA transcripts in conjunctival epithelia obtained from normal subjects ($n = 16$) and patients with DTS ($n = 19$). All data (mean $\pm$ SD) were compared with the normal control: * $P < 0.05$; ** $P < 0.002$.

Figure 3.

Correlation of tear MMP-9 activity in the study population ($n=64$) with (A) symptom severity score, $P < 0.001$; (B) SRI score, $P < 0.001$; (C) corneal fluorescein staining score, $P < 0.001$; (D) conjunctival fluorescein staining score, $P < 0.001$; (E) decrease in low-contrast visual acuity, $P = 0.002$; and (F) fluorescein TBUT, $P < 0.001$. r^2 = coefficient of determination.

Table 1

DTS Severity-Based Classification

Group	Symptom Severity Score	TBUT	Conjunctival Staining Signs [*]	Corneal Staining Signs [†]
Normal	20	>7 s	0	0
DTS1	>20	7 s	1 <3	<2
DTS2	>20	7 s	3	<6
DTS3	>20	7 s	3, mild conj. injection	6–11, filamentary keratitis
DTS4	>20	7 s	3, marked conj. injection; scarring	12, epithelial defect, ulceration

^{*} Obtained from the van Bijsterveld grading scheme.²⁵

[†] Obtained from the Baylor grading scheme.²⁶

Table 2

Clinical Features of DTS Patients and Controls

Group	Subjects (n)	Symptom Scores	SRI Score	TBUT (s)	Corneal Staining [*]	Conjunctival Staining	Schirmer I (min)
Control	18	16.59 ± 1.79	0.54 ± 0.10	9.56 ± 1.34	0	0	12.61 ± 7.62
DTS	46	35.70 ± 10.51	1.61 ± 0.82	5.55 ± 3.03	4.78 ± 5.22	1.43 ± 2.56	13.63 ± 9.69
DTS1	15	34.67 ± 12.67	1.48 ± 0.56	7.20 ± 3.00	0.73 ± 0.96	0	19.60 ± 9.22
DTS2	11	34.64 ± 10.34	1.25 ± 0.43	5.00 ± 2.19	3.00 ± 2.00	1.36 ± 2.25	16.64 ± 11.45
DTS3	9	33.63 ± 9.58	1.03 ± 0.70	5.56 ± 3.13	4.89 ± 4.43	1.22 ± 2.11	10.50 ± 6.57
DTS4	11	40.22 ± 8.41	2.54 ± 0.82	3.44 ± 2.70	12.00 ± 4.02	3.64 ± 3.53	6.11 ± 2.52

Data shown are mean ± SD.

^{*} Measured by Baylor grading scheme.¹⁹

Table 3**Ocular Surface Conditions in DTS Severity Groups**

DTS Severity Level	Condition
DTS1	Anterior blepharitis (1)
	MGD (3), MGD with rosacea (1)
	Prior LASIK (1)
	Conjunctivochalasis (5)
	Salzmann's nodular degeneration (1)
	Epithelial basement membrane dystrophy (2)
	Thyroid eye disease with lagophthalmos and decreased blink frequency (1)
DTS2	MGD (9)
	ATD without systemic disease (2)
DTS3	MGD (4), MGD with rosacea (1)
	Sjögren syndrome (SS) (1)
	Prior LASIK (1)
	Hypoesthesia following acoustic neuroma tumor excision (1)
DTS4	Blepharoconjunctivitis with punctal swelling (1)
	ATD without systemic disease (1)
	Stevens-Johnson Syndrome (SJS) (2)
	Sjögren Syndrome (SS) (6)
	Graft versus host disease (GVHD) (1)
	Radiation induced punctal stenosis (1)

Numbers in parentheses are the number of subjects with each condition. MGD, meibomian gland disease; ATD, aqueous tear deficiency.

Table 4

Tear MMP-9 Activity in Normal Control and DTS Groups

Group	MMP-9 Activity (ng/mL)
Normal (<i>n</i> = 18)	8.39 ± 4.70
DTS1 (<i>n</i> = 15)	35.57 ± 17.04 *
DTS2 (<i>n</i> = 11)	66.17 ± 57.02 *
DTS3 (<i>n</i> = 9)	101.42 ± 70.58 * [†]
DTS4 (<i>n</i> = 11)	381.24 ± 42.83 * [‡]

Data shown are the mean ± SD.

* *P* < 0.008 Compared with normal.[†] *P* < 0.003 Compared with normal and DTS1.[‡] *P* < 0.001 Compared with normal and the other DTS severity groups.