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Matrix Metalloproteinase 9 Testing in Dry Eye Disease Using a Commercially Available Point-of-Care Immunoassay

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Purpose: To measure matrix metalloproteinase 9 (MMP-9) in the tear film of patients with dry eye disease (DED) compared with controls and to correlate clinical findings.

Design: In a prospective study, 101 patients and controls underwent MMP-9 testing of the tear film. Thereafter, they were evaluated for symptoms and signs of DED.

Participants: Included patients were those who showed 3 of the following 4 dry eye criteria: ocular surface disease index (OSDI) score of more than 12, tear film break-up time (TBUT) of 10 seconds or less, Schirmer test results without anesthesia of less than 10 mm/5 minutes, and corneal staining results of 1 or more. Fifty-four healthy eyes and 47 eyes fulfilling diagnostic criteria for DED of various levels of severity were included in this study.

Methods: The tear film was analyzed for MMP-9 by a commercially available test (InflammaDry; Rapid Pathogen Screening, Inc, Sarasota, FL) detecting MMP-9 levels of more than 40 ng/ml. Symptoms and signs of DED were evaluated using the OSDI questionnaire, TBUT, conjunctival and corneal staining, Schirmer test results without anesthesia, and meibomian gland examination. These findings were correlated to results of the MMP-9 test in tears.

Main Outcome Measures: Positive MMP-9 results in tears.

Results: In 19 of 47 patients confirmed with dry eye (40.4%) and in 3 of 54 controls (5.6%), the MMP-9 results were positive. This difference was statistically significant ($P < 0.001$). Thus, the MMP-9 results indicated a clinically significant inflammation in 40% of dry eye patients. Positive results correlated well with subjective symptoms of DED evaluated by OSDI ($P = 0.001$), TBUT of less than 5 seconds ($P < 0.013$), Schirmer test results ($P < 0.001$), conjunctival staining ($P < 0.001$), and corneal staining ($P = 0.007$). Moreover, MMP-9 results correlated with the number of obstructed meibomian ducts ($P = 0.005$) and a pathologic meibomian gland secretion ($P = 0.001$). The MMP-9 results were increased significantly in women ($P < 0.001$) and in patients with autoimmune disease ($P = 0.005$), especially Sjögren's syndrome ($P = 0.001$) and thyroid disease ($P = 0.012$).

Conclusions: Matrix metalloproteinase 9 testing in DED is a valuable new diagnostic tool. It correlated well with other dry eye tests and identified the presence of ocular surface inflammation in 40% of confirmed dry eye patients. It may be especially helpful to identify patients with ocular surface inflammation and autoimmune disease and may facilitate the decision to institute anti-inflammatory treatment in these patients. *Ophthalmology 2016;■:1–9 © 2016 by the American Academy of Ophthalmology*

Matrix metalloproteinases (MMPs) are endopeptidases that play a fundamental role in many physiologic and pathologic processes in the eye. Matrix metalloproteinase 9 (MMP-9) participates in extracellular matrix remodeling after wounding of the corneal surface and has been implicated in the pathogenesis of sterile corneal ulceration, ocular allergy, fungal keratitis, burns, advanced keratoconus with irregular surface, active pterygia, conjunctivochalasis, blepharitis, and dry eye.[1–7] Matrix metalloproteinase 9 (gelatinase B) is produced by the corneal epithelium, fibroblasts, infiltrating leucocytes, and the lacrimal gland.[1,8,9] It is secreted as a proenzyme that is physiologically activated by other proteases. Active MMP-9 may be bound and inactivated by tissue inhibitors of MMPs.[1,3]

In dry eye, desiccating stress and hyperosmolarity are known to increase levels of MMP-9 significantly in corneal epithelial cells and tears of C57BL/6 mice,[4,9] probably by the JNK1/2, ERK 1/2, and p38 MAPKinase pathways.[9,10] Proinflammatory cytokines such as interleukin 1, tumor necrosis factor α, and tumor growth factor β1 are potent stimulators of MMP production. It seems that the interaction between these cytokines and MMPs creates a cycle of escalating inflammation on the ocular surface in dry eye.[11] Matrix metalloproteinase 9 promotes corneal extracellular matrix degradation and epithelial cell loss.[4] Knockout of MMP-9 alleviates the severity of experimental dry eye, indicating its role in pathogenesis. Moreover, MMP knockout leads to less alteration of corneal epithelial barrier

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function and lower levels of degraded tight-junction protein occludin compared with controls.[12] When tears and saliva of patients with Sjögren's syndrome were compared with those of healthy controls, MMP-9 was elevated significantly.[13] A quantitative MMP-9 assay detected an activity of 7.2±2.1 U/mg in normal control participants, which was significantly increased to 473.1±173.5 U/mg in patients with meibomian gland dysfunction (MGD; 66-fold; $P < 0.0001$) and to 651.7±208.3 U/mg in patients with Sjögren's syndrome (90-fold; $P < 0.0001$).[14] In another study, pro-MMP-9 levels were 23.61±17.4 ng/ml in controls compared with 58.56±30.1 ng/ml in blepharitis patients and 97.25±49.5 ng/ml in dry eye patients.[1] Conjunctival MMP-9 expression in impression cytologic specimens of patients with Sjögren's syndrome and MGD also were increased significantly compared with those in healthy controls. Topical corticosteroid treatment significantly reduced MMP-9 expression in both patient groups associated with amelioration of signs and symptoms.[15]

Matrix metalloproteinase 9 typically is measured in the laboratory by enzyme-linked immunosorbent assay, multiplex bead analysis, proteomic technology, or a combination thereof.[1,16,17] The InflammaDry (Rapid Pathogen Screening, Inc, Sarasota, FL), a new, disposable, single-use assay that provides a result in 10 minutes, allows MMP-9 testing in the office. It measures both active and latent MMP-9 and produces positive results when MMP-9 levels exceed 40 ng/ml. In a recent study, the InflammaDry showed a sensitivity of 85%, a specificity of 94%, a negative predictive value of 73%, and a positive predictive value of 97% to diagnose dry eye.[18] We evaluated the InflammaDry in a general population of dry eye patients compared with age-matched healthy controls.

Methods

This study was approved by the Ethics Committee of the Ludwig-Maximilians-Universität, München, Germany, and followed the tenets of the Declaration of Helsinki. Written consent was obtained from all participants after explanation of the protocol. One hundred one participants were included in this study. According to subjective symptoms and clinical tests, these persons were divided into 2 groups: 54 healthy controls and 47 patients with dry eye disease (DED) of varying degrees of severity. Patients who showed 3 of these 4 were included: Ocular Surface Disease Index (OSDI) score of more than 12, tear film break-up time (TBUT) of 10 seconds or less, Schirmer test results without anesthesia of less than 10 mm/5 minutes, and corneal staining results of 1 or more. Patients with diabetes mellitus, uveitis, or active ocular allergy; those receiving topical or systemic corticosteroid treatment or immunomodulatory therapy; those who had undergone ocular surgery in the previous 3 months; and those who wore contact lenses were excluded. Patients were stratified according to the criteria of Baudouin et al[19] into groups with mild or moderate disease and severe disease.

Clinical Tests

Patients underwent the following clinical procedures. The general medical history was recorded with special attention to collagen vascular and thyroid disorders. Current oral and topical medications were documented. Subjective symptoms were analyzed using the OSDI questionnaire. This is a self-administered 12-item questionnaire designed to provide rapid assessment of symptoms of ocular irritation consistent with DED, their impact on vision-related function, and environmental triggers. The OSDI answers sum up to a score as follows: (sum of the scores for all questions answered × 100)/(total number of questions answered × 4). The scale of the OSDI score from 1 to 100 allows grading with higher scores representing greater disability: normal, 0 to 12; mild disease, 13 to 22; moderate disease, 23 to 32; and severe disease, 33 to 100.[20] After testing Snellen visual acuity, MMP-9 in the tear film was evaluated using the InflammaDry in both eyes.

The InflammaDry uses direct sampling microfiltration technology. Matrix metalloproteinase 9, if present in the tear sample, is captured between MMP-9–specific monoclonal and polyclonal antibodies at a concentration of more than 40 ng/ml. The test was performed according to the InfammaDry package insert. No drops were placed in the patient's eye within 2 hours of conducting the test. In short, the eyelid was gently lowered to expose the palpebral conjunctiva. The sampling fleece was dabbed 8 to 10 times in multiple locations until the sampling fleece was saturated. The test was assembled by placing the fleece of the sample collector into the sample transfer window of the test cassette body. The absorbent tip was immersed into the buffer vial for 20 seconds and laid flat on a horizontal surface for 10 minutes. The test was read thereafter under brightly lit conditions and reread after 10 minutes for negative results as recommended by the manufacturer. Only test results showing a positive control line were evaluated. Test results were rated positive when a second—even faint—line appeared in the result zone. Semiquantitative interpretation using color intensity of the result line was not attempted. After a 10-minute interval, conjunctival hyperemia was evaluated at the slit lamp and graded from 0 (no hyperemia) to 3 (severe hyperemia).

Lissamine green conjunctival staining was conducted by using a lissamine-impregnated strip (Lissaver-Plus; Contopharma AG, Interlaken, Switzerland) wetted with saline. One drop of lissamine was instilled into the lower fornix, and staining was assessed using the Oxford grading scheme. In the Oxford grading scale, staining is represented by punctate dots on a series of panels (A–E). Staining

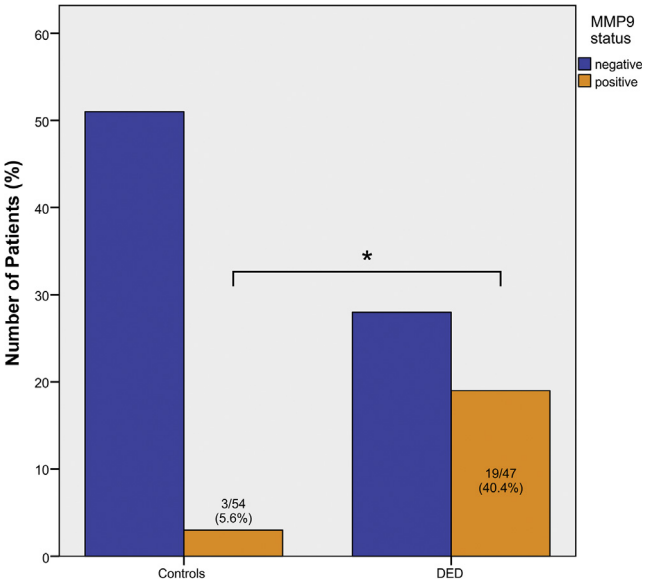

Figure 1. Bar graph showing positive matrix metalloproteinase 9 (MMP-9) results in patients with dry eye disease (DED) and controls. The difference between groups was statistically significant ($P < 0.001$). Asterisk indicates significant change.