American Academy of Ophthalmology
The Eye M.D. Association

Matrix Metalloproteinase 9 and Transglutaminase 2 Expression at the Ocular Surface in Patients with Different Forms of Dry Eye Disease

Pasquale Aragona, MD, PhD,[1] *M'Hammed Aguennouz, PhD,*[2] *Laura Rania, MD, PhD,*[1] *Elisa Postorino, MD, PhD,*[1] *Margherita Serena Sommario, MD,*[1] *Anna Maria Roszkowska, MD, PhD,*[1] *Maria Grazia De Pasquale, PhD,*[2] *Antonina Pisani, MD,*[3] *Domenico Puzzolo, MD*[3]

Objective: To evaluate the expression of matrix metalloproteinase 9 (MMP9) and transglutaminase 2 (TG2) in different forms of dry eye.

Design: Case control study.

Participants: Seventy-five female subjects divided into 3 groups: group 1, 15 healthy controls; group 2, 30 subjects with Sjögren syndrome (SS); and group 3, 30 subjects with Meibomian gland dysfunction (MGD).

Methods: A clinical assessment was carried out and impression cytologic specimens were processed for immunoperoxidase staining for MMP9 and TG2 and real-time polymerase chain reaction analyses were carried out for MMP9, TG2, interleukin-6, interferon-γ, B-cell lymphoma 2, and caspase 3. To study MMP9 and TG2 expression after anti-inflammatory treatment, patients were divided into 2 subgroups, one treated with saline and the other treated with saline plus topical corticosteroid eye drops (0.5% loteprednol etabonate) 4 times daily for 15 days. For statistical analysis, Student *t* test, Mann–Whitney *U* test, and Spearman's correlation coefficient were used as appropriate.

Main Outcome Measures: Conjunctival expression of MMP9 and TG2.

Results: MMP9 and TG2 expression were higher in both patient groups than in controls ($P < 0.0001$). Group 2 patients showed higher expression than group 3 ($P < 0.0001$). The Spearman's correlation coefficient showed in group 2 a positive correlation between MMP9 and TG2 expression ($\rho = 0.437$; $P = 0.01$), but no correlation in group 3 ($\rho = 0.143$; $P = 0.45$). Corticosteroid treatment significantly reduced MMP9 and TG2 expression in both groups, ameliorating symptoms and signs. A much higher percentage reduction was observed in SS.

Conclusions: The pathogenic mechanisms of the 2 forms of dry eye give an account for the different MMP9 and TG2 expressions in the 2 groups of patients. The higher expression in SS is determined by the direct autoimmune insult to the ocular surface epithelia, whereas in MGD patients, with an epithelial damage due to an unbalanced tear secretion, the molecules expression is significantly lower, although higher than in controls. The corticosteroid treatment induced a reduction of both molecules, although higher in SS than in MGD, because of its direct inhibitory effect on inflammation. *Ophthalmology 2015;122:62-71 © 2015 by the American Academy of Ophthalmology.*

The definition and classification of dry eye proposed by the Dry Eye Workshop states that dry eye is a multifactorial disorder in which inflammation plays a relevant role.[1] The pathogenic classification of dry eye includes 2 different forms of the disease: the first resulting from aqueous tear deficiency and the second from excessive tear evaporation.[1] Sjögren syndrome (SS) is the most representative cause of aqueous deficient dry eye, whereas Meibomian gland dysfunction (MGD) is the main cause of evaporative dry eye. The clinical features of these 2 different forms are quite specific in the mild forms of the disease, whereas they become almost superimposable in severe forms, when it becomes very difficult to distinguish the original pathogenic mechanism of the clinical pictures.[2,3]

Recent observations demonstrate that in dry eye pathogenesis an important role is played by both inflammation and apoptosis.[4,5] Matrix metalloproteinases are among the molecules released during inflammation. These are a family of zinc- and calcium-dependent enzymes involved in the breakdown of extracellular matrix in several physiological and pathologic processes. Matrix metalloproteinases play a crucial role in initiating and maintaining ocular surface damage.[6,7] Matrix metalloproteinase 9 (MMP9) is the most important gelatinase present on the ocular surface,[8] and its levels seem to be higher in tears of patients with dry eye.[6] Desiccating stress was found to increase MMP9 in a murine model.[9] Matrix metalloproteinase 9 acts on a large number of substrates, including the components of the corneal

http://dx.doi.org/10.1016/j.ophtha.2014.07.048
ISSN 0161-6420/14

epithelial basement membrane and the tight junction proteins ZO-1 and occludin. It thus may compromise most of the corneal epithelial barrier.[6] Therefore, the increased MMP9 activity in dry eye may contribute to the derangement of the corneal epithelial barrier, with increased corneal epithelial desquamation and corneal surface irregularity.[10]

A second group of molecules involved in ocular surface damage is represented by transglutaminases, ubiquitous proteins belonging to a family of calcium-dependent intracellular and extracellular cross-linking enzymes.[11,12] Transglutaminase 2 (TG2) is regulated by cellular stress, such as hydrogen peroxide treatment, or by external factors, such as ultraviolet radiations, and participates in the induction of cell death.[13] Hyperosmolarity and high intracellular calcium stimulate apoptosis in various cell types, leading to increased TG2 activity.[12] In fact, it was shown that where a necrotic rather than apoptotic cellular death occurs, there was a downregulated expression of TG2.[14] Transglutaminase 2 plays a role that ensures that, as soon as apoptosis has been initiated, it is completed without causing further inflammation and evident tissue injury. Transglutaminase 2 can promote apoptosis[11] by either direct mechanism or indirectly by promoting activation of transforming growth factor-β released by macrophages.[15] Experimental data indicate that corticosteroid treatment may influence the expression of MMP9 and TG2 in different tissues.[7,16]

The main goal of this study was to examine the expression of MMP9 and TG2 on conjunctival impression cytologic specimens in 2 different forms of dry eye and their variations after corticosteroid treatment. Furthermore, interleukin-6 (IL-6), interferon-γ (INF-γ), B-cell lymphoma 2 (Bcl-2), and caspase 3 were evaluated to check the epithelial status of inflammation and apoptosis.

Methods

Study Design

This was a single, masked, case-control study conducted at the Regional Referral Center for the Ocular Surface Diseases of the Department of Experimental Medical-Surgical Specialties at the University Hospital of Messina, Messina, Italy. Ethical approval was granted by the Ethics Committee of the University Hospital of Messina and the study was conducted in concordance with the tenets of the Declaration of Helsinki. Informed consent was obtained from all the participants after explanation of the nature and the possible consequences of the study. The main outcome measures were the conjunctival expressions of MMP9 and TG2 in the 2 dry eye forms studied. The secondary outcome measures were the changes in MMP9 and TG2 expression after corticosteroid treatment and signs and symptoms before and after corticosteroid treatment.

Participants

Seventy-five female subjects were included in the study and were divided into 3 groups: group 1, 15 healthy subjects as controls (mean age ± standard deviation, 53.9±11.7 years); group 2, 30 subjects affected by mild SS (mean age ± standard deviation, 56.3±8.8 years); and group 3, 30 subjects with mild MGD (mean age ± standard deviation, 57.3±13.8 years).

Inclusion criteria were patients with primary SS or MGD whose disease and therapy, systemic as well as topical, were stable for at least 1 month before the inclusion in the study; patients should have a symptom score higher than 2, in a scale grading from 0 to 3, in at least 2 symptoms; SS patients, diagnosed according to the American-European Consensus Group criteria (Table 1),[17] should have shown none or mild signs of lid involvement, characterized by mild lid margin hyperemia, meibomian gland expression with clear-cloudy fluid secretion on gland expression and an absence of lid changes, such as meibomian gland orifice atrophy,[18,19] or both, with a score lower than 2 according to the MGD grading system reported in Table 2,[20] whereas MGD patients should have had a score of 2 or higher. Exclusion criteria were: Schirmer I test results lower than 10 mm/5 minutes in the MGD group; systemic diseases other than SS or SS clinically changed in the last month; ocular diseases other than dry eye; and systemic or topical medications, or both, that could have interfered with tear production, such as β-blockers, benzodiazepines, hormones, and antihistamines.

Clinical Tests

Patients underwent the following clinical procedures, as previously described.[21] Each patient answered a questionnaire regarding ocular discomfort symptoms based on the following score: 0 = absent; 1 = mild; 2 = moderate; and 3 = severe. The following symptoms were investigated: burning, hitching, foreign body sensation, ocular dryness, tearing, photophobia, ocular pain, mucous discharge, and hyperemia. A total score (from 0 to 27), representative of the ocular discomfort degree, was obtained by adding up the scores of each symptom. Overall slit-lamp examination of the ocular surface was carried out under a dim light to avoid excessive tearing and without any manipulation of the lids.

Tear film break-up time (TBUT) was carried out as described by the National Eye Institute/Industry workshop.[22] In brief, from a fluorescein strip (fluorescein paper; Haag Streit AG, Köniz, Switzerland) wetted with saline, a drop was instilled into the lower fornix and the time gap between a complete blink and the appearance of a dark spot on the corneal surface, observed through a cobalt blue filter at the slit lamp, was recorded as the average of 3 consecutive measurements.

Fluorescein corneal staining was used to assess the cornea 3 minutes after TBUT evaluation. The stain was scored according to the National Eye Institute/Industry workshop criteria,[22] which divides the corneal surface into 5 areas. In each area, the staining pattern was graduated with a score ranging from 0 to 3, based on the confluence of stain: 0 = absent; 1 = isolated dots; 2 = confluent dots; and 3 = area with complete epithelial defect. The total score ranged from 0 to 15.

Lissamine green conjunctival staining was conducted by using a lissamine strip (Contacare Ophtalmics & Diagnostics, Gujarat, India), wetted with saline, to instill 1 drop into the lower fornix. Conjunctival surface, as suggested by the Collaborative Longitudinal Evaluation of Keratoconus study,[22,23] was divided into 4 areas: temporal, nasal, superior, and inferior. Each area was graduated with a score from 0 to 3 based on the confluence of stain, for a total score from 0 to 12.

The Schirmer I test was conducted using filter paper strips (SNO Strips; Laboratoire Chauvin, Aubenas, France) applied at the junction between the outer and middle third of the lower lid. The moisturized length was measured after 5 minutes. A moisture length of 10 mm or more was considered normal.

For MGD assessment, lid margins were studied at the slit lamp by expression of Meibomian glands and eversion for the evaluation of gland ducts and lid margins shape.