The Ocular Surface xxx (xxxx) xxx

Contents lists available at ScienceDirect

The Ocular Surface

journal homepage: www.elsevier.com/locate/jtos

Matrix metalloproteinase 9 positivity predicts long term decreased tear production

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ARTICLE INFO

Keywords:
Anti-inflammatory therapy
Diagnosis dry eye
Inflammatory dry eye
Matrix Metalloproteinase-9
Ocular surface inflammation
Tear production

ABSTRACT

Purpose: To investigate long-term correlations between Matrix Metalloproteinase-9 (MMP-9) testing and dry eye (DE) parameters. Additionally, to evaluate variability in MMP-9 results over time and with anti-inflammatory treatment.

Methods: Retrospective cohort study of DE patients with equal MMP-9 testing results (positive or negative) in both eyes and a minimum of 6 months of follow up. Our main outcome measure was to examine whether initial MMP-9 status affected change in DE parameters over time. Secondarily, we evaluated the frequency of MMP-9 status change over time and examined whether MMP-9 status change was impacted by treatment.

Results: 67 patients (76% female) fit the inclusion criteria. Mean age was 63 years with a mean follow up of 10.6 months. The majority (37/67, 55%) had concomitant systemic immune disease. MMP-9 testing was positive in both eyes in 39 individuals (58%) and negative in both eyes in 27 (42%) individuals. Of all DE parameters, initial MMP status predicted change in tear production. Individuals in the MMP-9 positive group had a greater decrease in production from baseline to final visit compared to the negative group (−2.6 vs 2.1, $P = 0.013$). In those initially MMP-9 positive, the frequency of becoming MMP-9 negative was higher in eyes treated with anti-inflammatory therapy compared to artificial tears (22.9% vs 3.3%, $P = 0.106$). However, only Lifitegrast 5% showed statistical significance compared to artificial tears (31.3% vs 3.3%, $P = 0.044$).

Conclusions: Eyes with detectable MMP-9 had significantly decreased tear production over time compared to those without detectable MMP-9. Anti-inflammatory treatment more frequently normalized MMP-9 compared to PFATs.

Dry eye (DE) disease is a multifactorial ocular surface disorder with a high global estimated prevalence that ranges from 5 to 50% [1]. Importantly, one of the key identified components in the pathophysiology of the disease is inflammation. Regardless of the inciting ocular surface stress, a key feature of DE includes hyperosmolarity which triggers a chain of inflammatory events that create a vicious circle perpetuating the DE cycle [2]. However, not all individuals with DE symptoms and signs have detectable inflammation on their ocular surface [3,4]. This suggests that in some patients, factors beyond inflammation drive disease.

Among the studied elements for ocular surface inflammation, a specific matrix metalloproteinase has been recognized as a biomarker for inflammation and developed into a point of care test. Matrix Metalloproteinase-9 (MMP-9) is an endopeptidase that is secreted into the tears and can break tight junctions of the ocular surface epithelium, resulting in loss of ocular surface barrier function. Most of the existing data on MMP-9 and its effect on the ocular surface is deduced from experimental DE mouse models [9–12]. These studies suggest that MMP-9 production is increased in DE and might contribute to corneal epithelial disruption [10]. In humans with DE, as compared to controls, MMP-9 levels were significantly elevated and increased with increasing DE severity, graded to the DEWS criteria. This study also found increased expression of the mmp-9 gene in the conjunctival epithelium of individuals with DE [13].

This data served as the basis for developing the InflammaDry® device (Quidel Corporation, San Diego, CA, USA), a single use assay that measures ocular surface MMP-9 levels in 10 min [14]. The assay results

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https://doi.org/10.1016/j.jtos.2020.10.003
Received 31 July 2020; Accepted 19 October 2020
Available online 21 October 2020
1542-0124/

in a dichotomous outcome, either as positive (levels above 40 ng/ml) or negative, although the intensity of the pink stripe relates to MMP-9 levels. InflammaDry® testing results vary between studies. Two studies found that the InflammaDry® had a sensitivity of 81–85% and specificity of 94–98% in DE patients compared to controls [15,16]. However, other studies have found that the minority of individuals with DE symptoms had detectable inflammation on the ocular surface (39–40.4%) [3,4]. Limitations of the above studies is that they are cross-sectional and as such, data on variability of testing with time is unknown. This is important as other diagnostic techniques for DE have shown significant temporal variation over time [17,18].

Given the importance of inflammation in DE, anti-inflammatories are often used in the management of disease. Due to the pivotal role of adaptative immunity in animal models of DE [5], namely $CD4^+$ T cells, several medications have been approved by the FDA that target this aspect of the immune system. Cyclosporine A 0.05% ophthalmic emulsion (Restasis, Allergan) and Cyclosporine 0.09% (Cequa, SUN) inhibit T-cell activation and consequently inflammatory cytokine production by selective inhibition of interleukins. This mechanisms is believed to be behind the increased tear production noted after commencement of therapy in individuals with DE [6,7]. Lifitegrast 5% ophthalmic solution (Xiidra; Novartis) inhibits T cell-mediated inflammation by blocking the binding of lymphocyte function-associated antigen 1 with intercellular adhesion molecule 1, and this mechanism is theorized to explain the reduction in symptoms and corneal staining noted after commencement of therapy in individuals with DE [8]. However, in all studies, not all individuals experienced decreased symptoms and/or improved signs with anti-inflammatory therapy, and it is not known which individuals will benefit most from a particular treatment.

Many gaps remain in the literature regarding the use of the InflammaDry as a point of care test. For example, it is not known whether MMP-9 positivity can serve as a marker to predict DE dynamics over time or a favorable response to anti-inflammatory therapy. As such, the purpose of this study was to measure DE measures over time in individuals with and without detectable ocular surface MMP-9 to evaluate whether MMP-9 can predict DE course. In addition, we evaluated the variability of sequential MMP-9 test results in our population, and compared the effect of medications (anti-inflammatory versus preservative free artificial tears (PFATs)) on MMP-9 results at follow up.

Methods

Study population

This study was a retrospective longitudinal chart review of patients evaluated at the Foster Center for Ocular Immunology of Duke University Eye Center between January 2018 and January 2020. The study was conducted in accordance with the declaration of Helsinki and Duke University Hospital Institutional Review Board approved this study with a waiver of informed consent due to the retrospective nature of this work.

The main inclusion criteria were DE patients that met 3 out of 4 following criteria: Ocular surface disease index (OSDI) score of more than 12, tear film break-up time (TBUT) of 10 s or less, Schirmer test results without anesthesia of less than 10 mm/5 min, and corneal staining results of 1 or more. Furthermore, these patients were required to have two MMP-9 tests, at least 6 months apart. MMP-9 was evaluated using InflammaDry® (Quidel Corporation, San Diego, CA) bilaterally by the same trained technicians. The technique consisted of dabbing the sampling fleece along the palpebral conjunctiva of the patient's lower eyelid until the device was saturated. Then, it was placed into the sample transfer window of the test cassette body. After this, the technician opened the buffer vial and immersed the absorbent tip. The test was read afterwards and reviewed after 10 min to determine final result.

Only test results showing a positive control line were evaluated. Test results were rated positive when a second line appeared in the result zone, regardless of the intensity [19]. That is, trace/borderline/faint values were considered as a positive test. To be included in the study, results of the initial test had to be equal in both eyes (i.e. both positive or both negative). Overall, 144 charts were reviewed and 77 individuals with baseline asymmetric MMP-9 results and/or with less than 6 months of follow up were excluded. This left 67 individuals who met inclusion and exclusion criteria and were subsequently divided into two groups: Individuals with baseline bilateral MMP-9 positivity and individuals with bilateral MMP-9 negativity.

Data collection, dry eye point of care tests

Demographic data was collected including age, sex and general medical history with special attention to the presence of systemic autoimmune or inflammatory conditions. Patients topical medication was documented. DE "point of care" tests were performed at multiple visits and included, in the order performed: ocular surface disease Index [20] (OSDI, Allergan) questionnaire, osmolarity (TearLab, San Diego, CA), InflammaDry, tear film break-up time (TBUT), corneal staining using the NEI grading score [21], and Schirmer's test without anesthesia measured at 5 min.

Statistical analysis

Statistical analysis was performed using SAS/STAT software, Version 9.4 of the SAS System for Windows. Copyright © 2002–2012 SAS Institute Inc. Our main outcome measures was to evaluate whether baseline MMP status predicted subsequent DE course. This was done using generalized estimating equations (GEE) that accounted for correlation between eyes. Second, we evaluated change in MMP-9 status from baseline to final visit via a McNemar's test (adjusted for the correlation between the two eyes) [22]. Third, in eyes that were initially MMP-9 positive, we compared the frequency of final MMP-9 positivity in eyes who used PFATs versus anti-inflammatory topical therapies using generalized estimating equations (GEE).

Results

Demographic Characterization and analysis of MMP-9 testing in Patients with ocular inflammation

67 individuals were included in the study with a mean age (±standard deviation, SD) of 63 ± 15 years; 51/67 (76%) were female and 16/67 (24%) male (Table 1). Average follow up was 10.6 months (range 6–23 months). MMP-9 testing was positive in 39/67 (58%) of patients in both eyes, whereas 28/67 patients (42%) tested negative for MMP-9 bilaterally at their initial consult. Systemic immune disease was detected in 37/67 patients (55%) and there was a trend for the MMP-9 positive vs negative group to have a higher frequency of concomitant

Table 1
Population demographics and clinical characteristics of matrix Metalloproteinase-9 (MMP-9) testing.

Characteristic	N = 134 eyes of 67 subjects
Age, mean ± SD (years)	63 ± 15
Mean Follow up ± SD (months)	10.6 ± 4.06
Sex, n (%)	51 (76.12%)
Female	16 (23.88%)
Male	
MMP-9 Status*, n (%)	39 (58%)
Positive	28 (42%)
Negative	
Associated systemic disease, n (%)	37 (55%)
Immune disease	30 (45%)
No immune disease	

*All patients had to present bilaterally equal MMP-9 result; therefore our population was constituted by 78 MMP-9 positive eyes and 56 MMP-9 negative eyes.

Immune diseases (64% vs 39%, $P = 0.084$). This difference was mostly driven by Graft Versus Host Disease (GVHD), which was significantly more frequent in MMP-9 positive vs negative eyes ($P = 0.031$) and represented the most prevalent condition in 14/37 of autoimmune patients (48%) (Fig. 1). Frequencies of Sjögren's and rheumatoid arthritis were similar between the groups.

MMP-9 positive testing Correlates with decreased tear production over time

The first study aim was to evaluate how baseline MMP-9 positivity influenced DE course. We found that tear production, as measured by Schirmer's testing, demonstrated a statistically significant decrease from initial to final measurements in MMP-9 positive compared to negative eyes (Table 2). Specifically, in the MMP-9 positive population, initial and final Schirmer's mean (±SD) value were 7.98 (±7.0 mm) and 5.38 (±6.63 mm), with a mean difference of −2.6 (±4.92 mm) ($P = 0.005$). This is compared to the MMP-9 negative population where initial and final mean (±SD) Schirmer's value were 8.72 (±6.52 mm) and 10.84 (±7.43 mm), with a positive mean difference of 2.12 (±8.21 mm) ($P = 0.171$). Comparison of mean difference in Schirmer values between the groups was statistically significant ($P = 0.013$). Therefore, MMP-9 positivity was associated with decreased tear production over time to a moderately low value, in comparison to the MMP-9 negative population, which did not experience a decline in production, over a 10.6 months mean follow up. Baseline MMP-9 positivity did not influence change over time of the other DE point-of-care tests studied (Table 2).

MMP-9 results show consistent repeatability during follow up

The initial proportion of MMP-9 positive eyes was 78/134 (58%). At final visit, the percentage of MMP-9 positive eyes increased to 90/134 (67%) while negative eyes decreased to 44/134 (33%) (Fig. 2). This was driven by 12/78 (15%) initially positive eyes becoming negative and 24/56 (43%) initially negative eyes becoming positive. The difference between proportions was not significant ($p = 0.081$).

Topical anti-inflammatory therapy normalizes MMP-9 expression in the tears of patients with inflammatory ocular surface disease

Of the 78 eyes initially positive for MMP-9, 32/78 (41%) eyes were treated with cyclosporine ophthalmic emulsion 0.05% (Restasis; Allergan), 16/78 (21%) with Lifitegrast 5% ophthalmic solution (Xiidra; Novartis) and 30/78 (38%) with PFATs. Overall, eyes treated with an anti-inflammatory were more likely to convert to MMP negativity compared to eyes treated with PFAT (22.9% vs 3.3%, $P = 0.106$), although the difference did not reach statistical significance. Eyes treated with Lifitegrast 5% had a statistically significant higher conversion from MMP-9 positive to negative (5/16, 31.3%) compared to eyes managed with PFATs (1/30, 3.3%) ($P = 0.044$). Eyes treated with Cyclosporine ophthalmic emulsion 0.05% also had a higher frequency of conversion from MMP-9 positive to negative in (6/32, 19%), but this did not reach statistical significance ($P = 0.166$) (Fig. 3). Interestingly, change in DE symptoms and signs did not relate to MMP-9 status (maintenance of positivity vs change to negative). Of note, individuals with a systemic immune disease were less likely convert to MMP negative compared to individuals without a co-morbid disease (3/41 eyes vs 9/37). However this difference was not statistically significant when accounting for inter-eye correlations (p = 0.13).

Table 2
Dry Eye point-of-care tests measurements per Matrix Metalloproteinase-9 (MMP-9) status.

DE Parameters	Initial Visit	Final Visit	*P* Value[a]
MMP-9 Positive Eyes			
OSDI	44.58 (±24.1)	42.34 (±25.7)	0.561
Osmolarity	304.6 (±21.0)	299.9 (±18.0)	0.154
Corneal Stain	3.76 (±5)	3.26 (±4)	0.550
TBUT	5.61 (±3.5)	4.66 (±3.6)	0.529
Schirmer's Test	7.98 (±7.0)	5.38 (±5.45)	0.005*
MMP-9 Negative Eyes			
OSDI	50.48 (±26.9)	46.44 (±27.5)	0.713
Osmolarity	302.6 (±16.0)	298.6 (±16.0)	0.369
Corneal Stain	3.19 (±4.2)	2.57 (±4.1)	0.439
TBUT	4.87 (±2.5)	4.55 (±2.5)	0.840
Schirmer's Test	8.72 (±6.52)	10.84 (±7.43)	0.778

Values are shown as mean ± standard deviation.
* Statistically significant differences.
Note: Tear production significantly decreased on MMP-9 positive eyes (P = 0.05). Initial measure on both groups were comparable (P > 0.05). Comparison of MMP-9 positive and negative eyes differences in values between visits presented a statistically significant difference (−2.6 vs 2.1, P = 0.013). Other dry eye mean ± SD initial final tests did not change significantly on both groups.
OSDI = Ocular Surface Disease Index Questionnaire; TBUT = Tear Break-Up Time. MMP-9 = Matrix Metalloproteinase-9.
[a] P-values test difference between means computed using generalized estimating equations to account for having both eyes in the analysis.

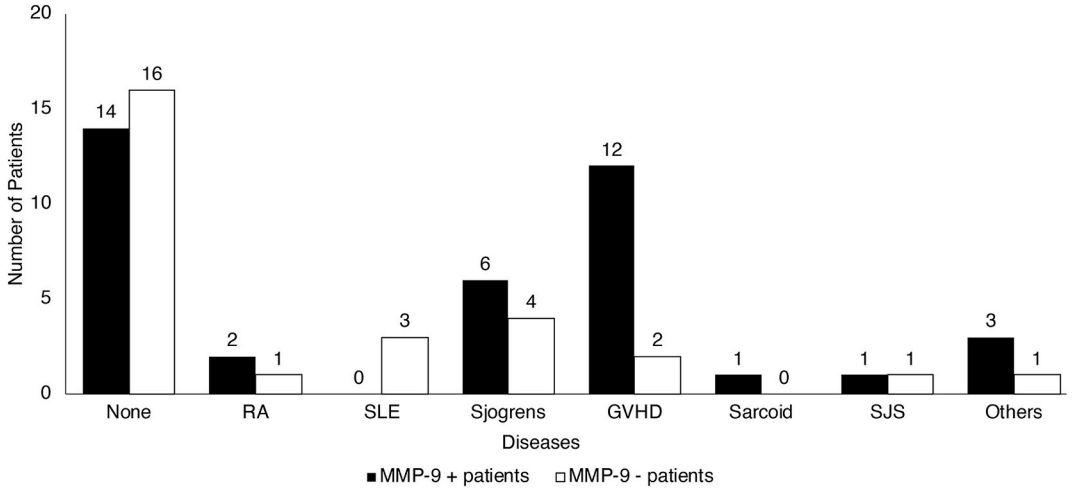

Fig. 1. Associated Immune Diseases per group. RA: Rheumatoid Arthritis. SLE: Systemic lupus erythematosus. GVHD: Graft Versus Host Disease, SJS: Stevens Johnsons Syndrome. There was a trend for higher frequency of concomitant systemic immune disease in the MMP positive group, with the difference being driven by GVHD, which was significantly higher in the MMP positive vs negative groups ($P = 0.031$).

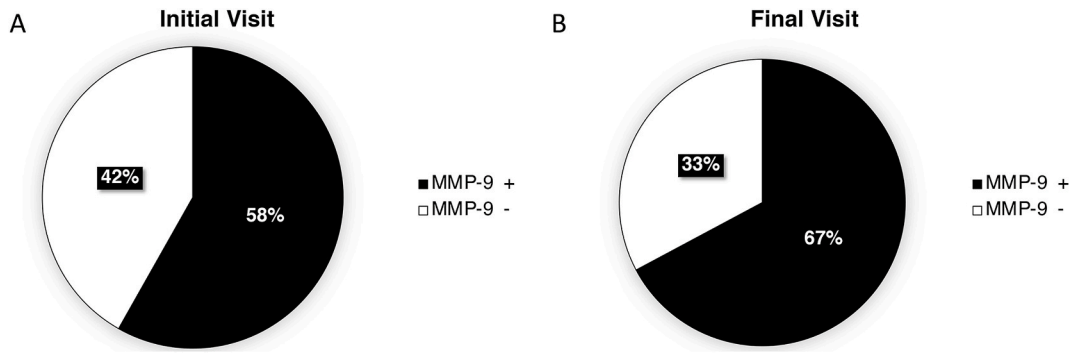

Fig. 2. Changes in MMP-9 Positivity Through Time. (A) Initial proportion of MMP-9 positive eyes was 78/134 (58%). (B) Final proportion of MMP-9 positive eyes was 90/134 (67%). The difference between proportions was not significantly different ($p = 0.081$).

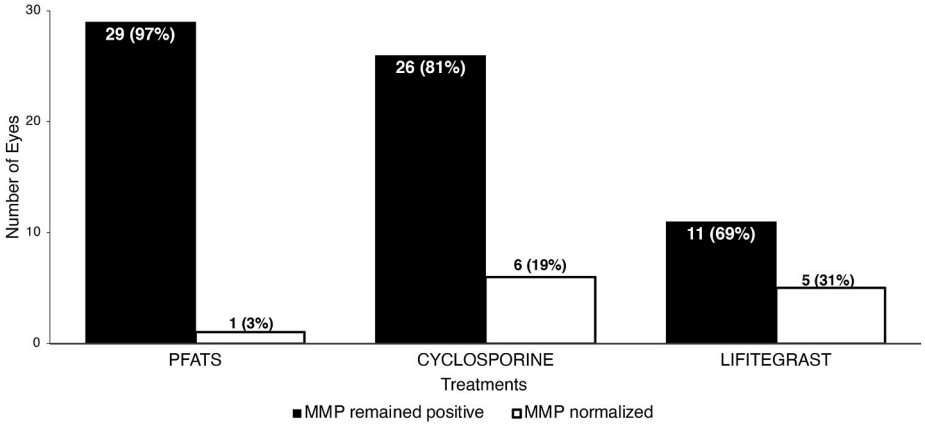

Fig. 3. MMP-9 normalization by treatment in eyes that were initially MMP-9 positive.
Lifitegrast 5% was associated with normalized MMP-9 in 5/16 (31.3%) of eyes, Cyclosporine 0.05% in 6/32 (18.8%) and PFATs in 1/30 (3.3%). Compared to PFATs, Lifitegrast 5% had a significantly higher conversion of normal ($P = 0.044$). Comparison of individual anti-inflammatory therapy against artificial tears was performed with adjusted Fisher's exact test using generalized estimations equations to account for having both eyes in the analysis.

Discussion

To summarize, we present a retrospective longitudinal study on a population of individuals with DE, the majority of whom had concomitant systemic immune conditions. MMP-9 was positive in 58% of our population, which is in the range of MMP-9 positivity rates published for other DE cohorts [3,4,15,16]. Most importantly, we found that initial MMP-9 positivity predicted decreased tear production over time as compared to MMP-9 negativity. Overall, results of MMP-9 testing was mostly stable, with the majority maintaining the same MMP-9 status (73%), while a minority converted from positive to negative (9%), or negative to positive (18%). However, this change was not associated with change in DE symptoms or signs. In the MMP-9 positive group, a higher frequency of eyes converted to MMP-9 negative after treatment with an anti-inflammatory agent (22.9%) as compared to artificial tears (3.3%), reaching statistical significance for Lifitegrast 5%.

We and others have previously examined cross-sectional associations between MMP-9 and tear production and did not find differences in Schirmer levels by MMP-9 status [3]. However, the novelty of our current work is the identification that MMP-9 positivity predicted decreased tear production over time. The relationship between inflammation and tear production is supported by a cross sectional study of 30 healthy volunteers that found an inverse correlation between MMP-9 levels (extracted from Schirmer strips, among other inflammatory molecules) and Schirmer's measurements ($r = -0.586$, $P < 0.001$) [23]. Interestingly, this study also found that TBUT and OSDI did not correlate to MMP-9 elevated levels, accentuating our study results.

Regarding MMP-9 modulation by anti-inflammatory therapy, when compared individually to artificial tears, both Cyclosporine 0.05% and Lifitegrast 5% reduced the frequency of MMP-9 positivity, although Lifitegrast 5% reached statistical significance over PFAT. Cyclosporine 0.05% and Lifitegrast 5% have been shown to reduce/normalize MMP-9 levels in prior studies with statistical significance. For example, topical cyclosporine 0.05% normalized MMP-9 (via Inflammadry) in 28% of eyes in a population of 30 individuals with signs or symptoms of DE over at least 1 months of treatment [24]. Our current study found a lower frequency of conversion from positive to negative (19%), perhaps due to the high frequency of co-morbid systemic immune diseases in our population. In another study, Lifitegrast 5% normalized MMP-9 values (via Inflammadry) in 38.9% of eyes in a population of 54 individuals with DE (21% of which had a co-morbid systemic immune disease) over a mean 91 day follow up [25]. This value is close to the 31.3% normalization frequency observed in our current study.

Another novel aspect of our study is the examination of how change in MMP-9 status impacted change in other DE parameters. Interestingly, we did not find a significant relationship between normalization of MMP-9 and improvement in symptoms. On another cross-sectional study, likewise we have not found differences in DE symptom severity by MMP-9 status [3]. This is an important finding to remember when judging effectiveness of anti-inflammatory medications. That is, using symptom improvement alone to judge an anti-inflammatory agent's success is likely not adequate and may cause premature termination of therapy with resultant negative consequences, such as decreased tear production. However, there is a lack of data on whether long term treatment with an anti-inflammatory can decrease the severity of ocular surface disease years down the line. This information is needed as the majority of DE studies evaluate the effect of anti-inflammatory therapy at 3 months while in the clinical arena, many individuals use such therapies for years.

It is also important to note that anti-inflammatory therapy did not normalize MMP-9 values in the majority of eyes (77%). Interestingly, eyes of individuals with a systemic immune disorder were less likely to

convert to MMP-9 negative compared to eyes in individuals without an immunologic diagnosis, although the findings were not statistically significant. This likely reflects the sensitivity of the InflammaDry as any value $\geq$ 40 ng/ml is read as positive. In some individuals, especially those with co-morbid immune conditions, starting levels of ocular surface inflammation may be much higher so that it is much more difficult to reach a negative value, even if the level of inflammation is decreased.

Beyond human data, animal studies also support the link between MMP-9 and decreased tear production over time. In mouse studies [26], desiccating stress has been shown to induce tear hyperosmolarity and inflammatory cytokine production. In addition, increased levels of ocular surface MMP-9 levels have been detected, with disruption of the corneal epithelium barrier, presumably by proteolytic cleavage of tight junctions [27]. MMP-9 presence also enhances the recruitment of lymphocyte T-Cells into the ocular surface and lacrimal glands [10], which further alters tear homeostasis. Auto reactive T-Cell Lymphocytic infiltration of the lacrimal gland further propagates the inflammatory cascade and leads to diminished reflex tear secretion [28], supporting our noted association between MMP-9 and diminished tear production.

As with all studies, our findings must be considering bearing in mild its limitations which include its observational retrospective design. To overcome these limitations, all the values presented as differences come from paired results only, and thus reflect a true variation of parameters. Furthermore, 55% of our population had concomitant systemic immune diseases, potentially limiting generalizability. Likewise, for statistical purposes we present data exclusively on individuals with bilateral symmetric MMP-9 testing results at initial presentation.

Despite these limitations, ours is one of the first studies examining whether MMP-9 status can be used as a biomarker for DE course over time. Our findings that initial MMP-9 positivity predicted decreased tear production over time may be used clinically to identify individuals that should be monitored more carefully and potentially be treated with anti-inflammatory therapy. In addition, the observation that MMP-9 normalization was not related to change in DE symptoms or signs suggests that it is important to re-examine definitions for treatment success. Prospective studies with diverse populations will be needed to assess repeatability and impact of our findings.

Disclosure

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Financial Support

Supported by the Department of Veterans Affairs, Veterans Health Administration, Office of Research and Development, Clinical Sciences Research I01 CX002015 (Dr. Galor), Biomedical Laboratory R&D (BLRD) Service I01 BX004893 (Dr. Galor), Department of Defense GW190010 (Dr. Galor), R01EY026174 (Dr. Galor), NIH Center Core Grant P30EY014801 and Research to Prevent Blindness Unrestricted Grant (Dr. Galor). National Institutes of Health/National Eye Institute: R01EY030283 (Dr. Perez), R41EY029185-01 (Dr. Perez), R01EY024485 (Dr. Perez), Duke NIH Center Core Grant and Duke Research to Prevent Blindness Unrestricted Grant (Dr. Perez).

Financial Disclosures

M.S: None, H.M.M: None, S.S.S: None, A.G: Novaliq, Dompe, Novartis, V.L.P: Alcon, Eyegate, Novaliq, Trefoil, Quidel, OBTears.

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