

Role of Matrix Metalloproteinase-9 in Diagnosis of Dry Eye

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Abstract

Background: Dry eye is a “multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface”. Symptoms of dry eye, which include visual disturbances and pain/dysesthesias, have been found to negatively impact quality of life.

Aim of Study: To assess the presence of ocular surface Matrix Metalloproteinase-9 (MMP-9) in dry eye by InflammDry® test.

Patients and Methods: A prospective cross-sectional study included a total of 40 eyes with dryness. This study was involved patients visiting outpatient clinic in Qalawoon Hospital from December 2018 till May 2019. The tear film was analyzed for MMP-9 by InflammDry® test, symptoms and signs of dry eye disease were evaluated using the Ocular Surface Disease Index (OSDI) questionnaire, Tear Breakup Time (TBUT), corneal staining, Schirmer and Meibomian gland examination.

Results: Mean age of the study population was 39.45 years \pm 9.06 SD (range 30-60), sixty five percent of study population were females. Mean of Ocular Surface Disease Index was 23.05 \pm 5.00 SD (14-33 range), Schirmer test mean was 4.60mm/5 minutes \pm 2.11 SD (1-8mm/5min range), Tear Breakup Time mean was 5.40 seconds \pm 1.61 SD (3-9 range) and corneal staining mean was 2.45 \pm 0.68 SD (2-4 range). Seventy five percent of the study population had Meibomian gland dysfunction. There was a highly statistically significant correlation between InflammDry® results and Meibomian glands dysfunction. There were statistically non-significant correlations between Ocular surface disease index, Schirmer test results, tear breakup time, corneal staining and positive results of InflammDry® test.

Conclusion: There is direct correlation between inflammation and dry eye disease. Also, Matrix metalloproteinase testing in dry eye disease is a valuable new diagnostic tool to identify the presence of ocular surface inflammation.

Key Words: Matrix Metalloproteinase-9 – Dry eye.

Introduction

DRY eye is a “multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface” [1].

Symptoms of dry eye, which include visual disturbances and pain, have been found to negatively impact quality of life [2].

Due to the multifactorial etiology of dry eye and its varied pathophysiologic mechanisms, it has been difficult to identify specific biomarkers that can aid in the diagnosis of dry eye. Inflammation is known to play an important role in the initiation and propagation of dry eye [3].

Matrix Metalloproteinase-9 (MMP-9) is a 23 zinc and calcium ion-dependent enzyme important for tissue remodeling in normal physiological processes like wound healing and bone development. It plays a pathogenic role in inflammatory diseases, arthritis, cardiovascular diseases, pulmonary diseases, and cancer [4].

MMP-9 activity is regulated by epigenetic processes, cell-cell interactions, and cytokine-mediated pathways. On the corneal surface, the hyperosmolarity of the tear fluid seen in dry eye has been shown to trigger the Stress-Activated Protein Kinase (SAPK) signaling cascade. SAPK signaling leads to the release of MMP-9 from corneal epithelial cells themselves, thus initiating a cycle of progressive inflammation. Tight junction proteins; occludin and Zonula Occludens-1 (ZO-1) are cleaved by MMP-9, thereby disrupting epithelial layers [5].

T-cell recruitment, the proteolytic activity of the MMP-9 molecule itself, and activation of se-

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cretion of additional cytokines initiate a self-perpetuating cycle of inflammation, secretory dysfunction, and worsening eye dryness [6].

In this study, we will use InflammDry (RPS, Sarasota, FL), a novel test to measure level of MMP-9. It measures the presence of MMP-9. The test provides a qualitative (yes/no) response. The lower detection limit of the test is 40ng/ml. If the collected sample is less than 5 μ L, the test may falsely give a negative result [7].

Aim of the work:

To assess the presence of ocular surface Matrix Metalloproteinase-9 (MMP-9) in dry eye by InflammDry® test.

Patients and Methods

A prospective cross-sectional study conducted on 40 eyes of forty patients attending outpatient clinic in Qalawoon Hospital from December 2018 till May 2019. The study was conducted on patients with dry eye. Exclusion criteria included; contact lens wearer, previous refractive, cataract, glaucoma and retinal surgery within last six months, collagen vascular disease, HIV, sarcoidosis, pulmonary and cardiac diseases, graft-versus host disease, history of cancer therapy either chemotherapy or radiotherapy and history of using ocular medications as steroids & anti-glaucoma medications. The tear film was analyzed for MMP-9 by InflammDry® test. Symptoms and signs of dry eye disease were evaluated using the OSDI questionnaire, TBUT, corneal staining, Schirmer and Meibomian gland examination.

Statistical analysis:

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 23. The quantitative data were presented as mean, standard deviations and ranges when their distribution was found parametric. Also, qualitative variables were presented as number and percentages. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p -value <0.05 was considered significant.

Results

This study was conducted on 40 eyes of 40 patients with dry eye disease, 65% of the study populations were females and 35% were males. The mean age was 39.45 years \pm 9.06 SD (30-60 years range) as shown in (Table 1).

Table (1): Demographic data of the study group.

	No.=40
Age (years):	
Mean \pm SD	39.45 \pm 9.06
Range	30-60
Sex:	
Female	26 (65.0%)
Male	14 (35.0%)

No: Number.
SD: Standard Deviation.

In this study we found non-significant correlation between the age and sex of study group and inflammaDry® test results whether the results were negative or positive as shown in (Table 2).

Table (2): Correlation between inflammaDry® test results and demographic distribution of study population.

	InflammaDry® test for matrix metalloproteinase-9		Test value	p -value	Sig.
	Negative No.=4	Positive No.=36			
Age (years):					
Mean \pm SD	39.50 \pm 2.89	39.44 \pm 9.52	0.011*	0.991	NS
Range	37-42	30-60			
Sex:					
Female	4 (100.0%)	22 (61.1%)	2.393*	0.122	NS
Male	0 (0.0%)	14 (38.9%)			

No: Number. *: Chi-square test.
SD: Standard Deviation. •: Independent t -test.
 p -value >0.05: Non-Significant (NS).

Table (3): Relations between results of inflammaDry® and other diagnostic tests for dry eye disease.

	InflammaDry® test for matrix metalloproteinase-9		Test value•	p -value	Sig.
	Negative No.=4	Positive No.=36			
Ocular surface disease index:					
Mean \pm SD	25.50 \pm 0.58	22.78 \pm 5.20	1.035	0.307	NS
Range	25-26	14-33			
Schirmer test (mm/5min):					
Mean \pm SD	4.00 \pm 1.15	4.67 \pm 2.19	-0.595	0.556	NS
Range	3-5	1-8			
Tear breakup time test (sec):					
Mean \pm SD	4.00 \pm 0.00	5.56 \pm 1.63	-1.888	0.067	NS
Range	4-4	3-9			
Corneal staining:					
Mean \pm SD	2.50 \pm 0.58	2.44 \pm 0.69	0.154	0.879	NS
Range	2-3	2-4			

No : Number.
SD : Standard Deviation.
mm : Millimeter.
min : Minutes.
sec : Seconds.
 p -value >0.05: Non-Significant (NS).
• : Independent t -test.

There were statistically non-significant correlations between ocular surface disease index and InflammADry® test results. As well as there were statistically non-significant correlations between Schirmer test and results of InflammADry® test. We found also that there were statistically non-significant correlations between tear breakup time and results of InflammADry® test. Also, the correlations between corneal staining and results of InflammADry® test are statistically non-significant as shown in (Table 3).

Among the study group there were thirty eyes have Meibomian gland dysfunction and positive results of InflammADry® test. Also, we found six eyes have normal Meibomian glands and positive results. The relation between Meibomian glands dysfunction and results of InflammADry® test is highly significant with *p*-value <0.01 as shown in (Table 4).

Table (4): Relation between Meibomian gland assessment and results of InflammADry® test.

Meibomian gland assessment	InflammADry® test for matrix metalloproteinase-9				Test value*	<i>p</i> -value	Sig.
	Negative		Positive				
	No.	%	No.	%			
• Normal	4	100.0	6	16.7	13.333	0.000	HS
• Meibomian gland dysfunction	0	0.0	30	83.3			

No: Number.

p-value <0.01: Highly Significant (HS).

*: Chi-square test.

Discussion

Dry eye is often underdiagnosed because of the poor correlation between commonly used objective signs and symptoms and the variability of signs over time [8].

Matrix metalloproteinase 9 is an inflammatory biomarker that has been shown to be elevated in the tears of dry eye patients. The possibility to detect elevated MMP-9 levels accurately in the tear film may lead to earlier diagnosis and improved treatment of ocular surface disease [9].

This study included 40 eyes visiting the outpatients clinic of Qalawoon Eye Hospital, Cairo, who satisfied the inclusion and exclusion criteria between December 2018 and May 2019 aiming to determine MMP-9 in dry eye disease.

This study showed that 90% of patients with dry eye disease had InflammADry positive results which detect presence of MMP-9. This high per-

centage results can be explained by various factors like effect of environment, humidity and stress which can increase level of MMP-9 in tear film as reported by Teson et al., [10].

Sambursky et al., [7] published the sensitivity and specificity of a point-of-care MMP-9 immunoassay (InflammADry), in their prospective study incorporating 143 patients with dry eye and 63 controls, 85% of patients with dry eye had positive InflammADry results and elevated MMP-9, and 6% of the control group had positive results.

Sambursky et al., [3] in another prospective study reported that 81 % of patients with dry eye had InflammADry positive results. Also, Sambursky [11] in a retrospective study on 100 patients reported that 60% of the patients with dry eye symptoms tested positive for elevated MMP-9.

However, Lanza et al., [4] reported that 39% of participants with dry eye symptoms showed positive results for MMP-9 using the InflammADry. Because the InflammADry shows positive results only when the MMP-9 level in the tears is more than 40ng/ml, most of the study patients would have shown negative results. Lanza assumed that dry eye patients tested in recent studies belong in this gray zone.

Messmer et al., [12] who conducted a study on 47 patients with dry eye and 54 controls, found that 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.

This study showed a female predominance (65.0%), it correlates with other studies as Messmer et al., [12] who found a female predominance 66.0% and Sambursky [11] who found that female patients represented 72% of the study population.

On the other hand, Lanza et al., [4] reported that male patients represented 90% of the study population.

This difference in female/male ratio can be explained in part by their greater percentage in the population.

This study showed that there is a highly significant correlation between MMP-9 and Meibomian gland dysfunction. This coincides with other studies as Aragona et al., [5] and Messmer et al., [12] who showed that MMP-9 positive results correlated

with the number of obstructed Meibomian ducts and a pathologic Meibomian gland secretion.

While, Lanza et al., [4] found that there was a statistically non-significant correlation between MMP-9 on the ocular surface and MGD whether patients were positive or negative.

In this study a statistically non-significant correlation was found between MMP-9 and subjective symptoms evaluated by OSDI. This result coincides with that found in other studies as Schargus et al., [13], Aragona et al., [5] and Lanza et al., [4].

While Messmer et al., [12] showed that positive results of InflammDry test correlated well with subjective symptoms of dry eye disease evaluated by OSDI. Possible explanation for the discordance between dry eye symptoms and elevated MMP-9 may be the intermittent nature of mild dry eye disease, which leads to symptoms only at the time of an environmental stress. Also, OSDI is a subjective method to evaluate symptoms of dry eye.

In this study also, we found a statistically non-significant correlation between MMP-9 positive results and Schirmer test results. This result coincides with that found in other studies as Schargus et al., [13], Aragona et al., [5] and Lanza et al., [4].

However, Messmer et al., [12] showed that there was a statistically significant correlation between positive results of InflammDry test and Schirmer test results.

In this study also, a statistically non-significant correlation was found between MMP-9 positive results and TBUT. This correlates with that reported by Schargus et al., [13] and Lanza et al., [4].

While, Messmer et al., [12] showed that positive results of InflammDry test correlated well with TBUT.

In this study also, there were a statistically non-significant correlation between MMP-9 positive results and corneal staining. This result coincides with that reported by Lanza et al., [4].

However, Schargus et al., [13] and Messmer et al., [12] showed significant correlation between results of MMP-9 and corneal staining. This variation may have occurred because of the disadvantage of staining, as dry eye cannot be clinically differentiated from other conditions that lead to ocular surface staining such as poor lid apposition and medication toxicity.

Conclusion:

There is direct correlation between inflammation and dry eye disease. Also, Matrix metalloproteinase testing in dry eye disease is a valuable new diagnostic tool to identify the presence of ocular surface inflammation.

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دور الماتريكس ميتالوبروتينيز-٩ في تشخيص مرض جفاف العين

جفاف العين هو مرض متعدد العوامل يؤثر على الدموع وسطح العين ويؤدي إلى أعراض عدم الراحة، وإضطراب البصرية، وعدم استقرار الدموع على سطح العين.

في هذه الدراسة المستعرضة، سوف نتناول مفتاح للسؤال المهم: هل يلعب الإلتهاب أى دور في الآلية الفيسيولوجيا والمرضية المسببة لجفاف العين؟ لذلك، قمنا بتقييم وجود إنزيم "الماتريكس ميتالوبروتينيز-٩"، وهو الإنزيم الذى يلعب دوراً أساسياً فى الإلتهاب.

خضع جميع المشاركين لتقييم مؤشر الأمراض السطحية للعين، وإختبار شيرمر، وإختبار وقت تكسير الدموع، وصبغة القرنية وإختبار "الإنفلاما دراى" الذى يكشف عن وجود إنزيم "الماتريكس ميتالوبروتينيز" فى الدموع.

تم إجراء هذه الدراسة على أربعين عين بها مرض جفاف العين. وكان متوسط عمر المرضى المشمولين فى هذه الدراسة هو 39.45 ± 0.6 سنة. وكانت الغالبية العظمى من الإناث بنسبة ٦٥٪ من مجموع المرضى المشاركين.

فى هذه الدراسة وجدنا أن هناك علاقة ذات دلالة إحصائية هامة بين خلل غد "ميبومين" والنتائج الإيجابية لإختبار "الإنفلاما دراى".

وقد وجدنا أيضاً علاقة ليست ذات دلالة إحصائية هامة بين النتائج الإيجابية لإختبار "الإنفلاما دراى" وكل من مؤشر الأمراض السطحية للعين وإختبار شيرمر ووقت تفكك الدموع ونتائج صبغة القرنية.

فى هذه الدراسة وجدنا علاقة ليست ذات مغزى إحصائية هام بين عمر وجنس مجموعة الدراسة ونتائج إختبار "الإنفلاما دراى" سواء ما إذا كانت النتائج سلبية أو إيجابية.