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Importance of estimation of inflammatory marker (MMP 9) in tear film of dry eye patients

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Abstract

Aim: To evaluate the presence of inflammatory marker MMP-9 in dry eye patients and institute appropriate anti-inflammatory therapy.

Materials and Methods: This is a prospective study done in 30 patients, suspected of having dry eye based on history and clinical examination inputs. All of them followed the following sequence - Meticulous History, Clinical examination, Ocular Surface disease index (OSDI) score, Tear film break up time (TBUT), Schirmer I test, Tear osmolarity level with Tearlab and MMP- 9 estimation with Inflamma Dry® Package Insert.

Results: Out of 30 patients, Female (56.66%) dominated over male patients(43.33%). The most common age group in the study was between 51 and 60 years while it was nil in age group below 20 years. Ocular Surface Disease Index(OSDI) score graded the patients in Mild (23.33%), Moderate (63.33%) and Severe(13.33%) respectively. We took 315mOsms/l as a cut off value for tagging the tear as hyperosmolar and 17(56.66%) patients fell in this category. MMP-9 was found to be positive in 9 (30.00%) patients. Patients having inflammatory marker positive in tear film, were put on appropriate anti-inflammatory therapy.

Discussion: Elevated tear fluid osmolarity has been recognised as a common feature of dry eye for decades 14. Tear osmolarity is one of the best single metrics to diagnose and classify dry eye disease 13. Hyperosmotic stress stimulates the expression and production of inflammatory markers including MMP- 9 by human corneal epithelial cells(HCEC)15. In the present study we evaluated the MMP- 9 level in 30 dry eye patients and found 9 cases exhibiting positive response. Osmoprotectants (L- carnitine, Erythritol, Betaine and Glycerol) differentially suppress gene expression, protein production, and enzymatic activity of inflammatory markers in primary HCECs exposed in hyperosmotic stress16. In light of above evidence we prescribed a tear substitute having the osmoprotectants as its ingredients, to our MMP- 9 positive dry eye patients. In addition we added anti-inflammatory agents e.g. topical soft steroids and topical Cyclosprine as per the DEWS treatment recommendations.

Keywords: Hyperosmolarity, MMP-9 and Osmoprotectants

Introduction

Dry eye is a multifactorial disease of the tears and ocular surface with symptoms of discomfort, visual disturbance and tear film instability with potential damage to the ocular surface. It is often accompanied by increased osmolarity of the tear film and inflammation of the ocular surface.(1) Prevalence of dry eye is 5–34% depending on the criteria for Dry Eye Disease (DED) applied, the population studied, and geographic location.(2-6)

Matrix Metalloproeinases (MMPS): a) Are a family of neutral zinc proteases that degrade extracellular components including the triple- helical structure of collagen. b) Synthesised and secreted by multiple cell types, including corneal epithelial cells and fibroblasts. c) Implicated in a wide range of physiologic and pathological processes.(7) d) Hyperosmolarity of tear film is a potent proinflammatory stress, which stimulates the expression and activity of matrix metalloproteinases (MMPs) in ocular surface epithelium.(8,9)

DED has a significant negative impact on quality of life due to chonic irritation and pain,(10) which can have a negative impact on visual performance and ability to perform daily tasks (e.g., reading, driving).(11) These detrimental effects on functioning may lead to anxiety and depression.(12)

Aims and Objectives

To evaluate the presence of inflammatory marker MMP-9 in dry eye patients and institute appropriate anti-inflammatory therapy.

Materials and Methods

This is a prospective study done in 30 patients, suspected of having dry eye based on history and clinical examination inputs.

All of them underwent the following – Meticulous History, Clinical examination, Ocular Surface Disease Index (OSDI) score, Tear film Break up Time (TBUT), Schirmer I test, Tear Osmolarity level (Tear Lab) and MMP-9 estimation with Inflamma Dry® Package Insert. Minimum 2 hour gap between Tear lab and MMP 9 test was religiously maintained. It was ensured that no manipulation of eye took place in the 2 hour interval.

Results

Sex distribution: Female patients(56.66%) dominated their male counterparts(43.33%) in our present study.

Table 1: Showing Sex Distribution

Sex	Number (N=30)
Male	13 (43.33%)
Female	17(56.66%)

Age distribution in the study: The most common age group was between 51 to 60 years of age while it was nil in age group below 20 years. This reflects the view that the dry eye disease icreases with age.

Table 2: Showing age distribution

Age group	Number (N= 30)
0 - 10	Nil
11- 20	Nil
21- 30	2(6.66%)
31- 40	5(16.66%)
41- 50	9(30.00%)
51- 60	11(36.66%)
61- 70	3(10.00%)

Ocular Surface Disease Index (OSDI) score was used to categorise the selected patient's grade of dry eyes. Accordingly mild, moderate and severe group constituted in our study are 23.33%, 63.33% and 13.33% respectively.

Table 3: Showing grade of dry eye as per OSDI score

Grade of dry eye as per OSDI index	Number of patients (N= 30)
Mild	7 (23.33%)
Moderate	19(63.33%)
Severe	4(13.33%)

Tear film Break Up Time (TBUT): We considered < 10 seconds as Positive / Significant and suggestive of dry eye. 17(56.66%) out of total 30 patients are found to have positive TBUT.

Indeterminate group constitutes those patient who could not carry out the test satisfactorily.

Table 4: Showing result of TBUT

Interpretation	Number of patient (N= 30)
Positive (TBUT < 10 SEC)	17 (56.66 %)
TBUT > 10 Sec	8 (26.66%)
Indeterminate	5 (16.66%)

Results of the schirmer I score: 13(43.33%) of cases showed Schirmer I score less than 10 seconds and suggestive of dry eyes. However 9 cases had Schirmer more than 15 seconds which is probably due to reflex watering.

Schirmer I Score (In mm)	Number of patient (N = 30)
< 10	13(43.33%)
10- 15	8(26.66%)
15-20	5(16.66%)
20- 25	4(13.33%)

Tear film Osmolarity was calculated with Tear Lab Osmolarity.

We took 315 mOsms/l as the normal cut off as specified by the Manufacturer. Anything above was tagged as Hyperosmolar tear in our study.

Table 5: Showing Tear film Osmolarity

Tear film Osmolarity (mOsms/l)	Number of Patients (N =30)
> 315	17 (56.66%)
315- 310	10 (33.33%)
< 310	3 (10%)

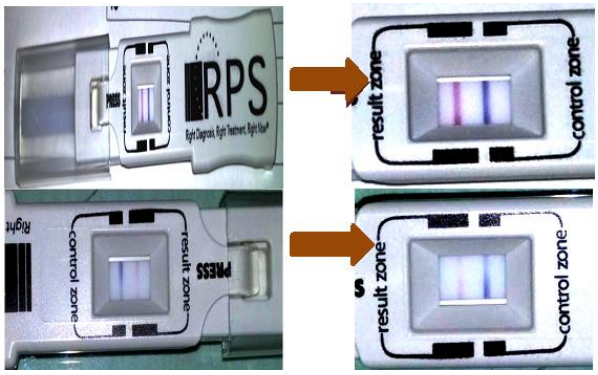
MMP-9 Estimation: In our study we found 9(30%) patients whose MMP-9 level was positive i.e. > 40 ng/ml. This suggested that inflammatory pathology was present in those patients and called for specific therapy.

Table 6: Showing MMP-9 result

Interpretation of result	Number of patient (N = 30)
Positive (> 40 ng/ml)	9(30%)
Negative (< 40 ng/ml)	21(70%)

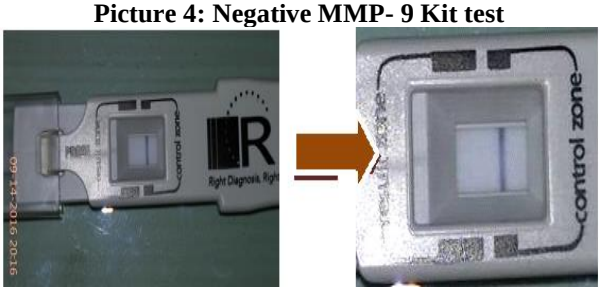
Results of MMP-9 Test

Picture 3: Positive MMP- 9 Kit test



21 (70%) of the patients exhibited negative response i.e. MMP- 9 < 40 ng/ml.

Picture 4: Negative MMP- 9 Kit test



Patient with associated conditions that are likely to play role in pathogenesis of dry eye, found in our study and corresponding MMP- 9 levels are as follows:

Associated conditions	Number of patient	MMP -9 level
Post-Menopausal women	9	2
Post-Surgery (Lasik, cataract)	10	Nil
Long hours of Computer work (> 5 hours /day for 5 days/week.	11	1
Thyroid dysfunction	8	2
Connective tissue disorder (AS, RA)	7	4

The above chart clearly shows that the percentage of tear film MMP-9 positive is highest in patients suffering from connective tissue disorders while it is least in Post-Surgery patients in our study.

We provided our patients with the printed record. Follow up at 3 months.

Discussion

Tear osmolarity is one of the best single metrics to diagnose and classify dry eye disease.[13] In our study we have taken 315 mOsmsl/l as a cut off as specified by the manufacturer. However the value is arbitrary and the dry eye fraternity is yet to come up with a definitive value.

In the present study we evaluated the MMP- 9 level in 30 dry eye patients and found 9 cases exhibiting positive response. Elevated tear fluid osmolarity has been recognised as a common feature of dry eye for decades.[14] Hyperosmotic stress stimulates the expression and production of inflammatory markers including MMP- 9 by human corneal epithelial cells(HCEC).[15]

Osmoprotectants (L- carnitine, Erythritol, Betaine and Glycerol) differentially suppress gene expression, protein production, and enzymatic activity of inflammatory markers in primary HCECs exposed in hyperosmotic stress.[16]

They may have potential effects in protecting ocular surface epithelia from MMP- mediated disorders in dry eye disease.[16]

In light of above evidence we prescribed a tear substitute having the osmoprotectants as its ingredients, to our MMP- 9 positive dry eye patients. In addition we added anti-inflammatory agents e.g. topical soft steroids and topical Cyclosprine as per the DEWS treatment recommendations. Tetracyclines and Omega 3 fatty acids were added wherever indicated.

Conclusion

Dry Eye Disease (DED) is therefore not simply a lack of tears, but a complex surface disease in which the tear film is unbalanced and no longer provides sufficient nourishment or protection to the ocular surface (reviewed in 2007 Report of the International Dry Eye Workshop (DEWS[17]).

Identification of inflammatory markers in tear surface therefore, has paramount importance in dry eye patients, so that effective and definitive intervention can be taken to restore cell volume, stabilise protein function and protect the ocular surface cells from hyperosmolarity stress.

Last but not the least, it can be inferred from the above discussion that all these newer diagnostics and appropiate intervention are likely to improve the quality of life of the dry eye patients in the coming years.

However longer follow ups are required to throw more light on the subject.

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