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Role of Inflammation and Infectious Disease As an Etiological Cause in Patients with Obstruction of the Tear Drainage Pathway

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***Abstract*—** Several eye diseases such as Conjunctivitis, Dry eye, Blepharitis, Uveitis, Diabetic Retinopathy, Cataract etc. affect people on a daily basis. The etiological cause behind a few of these diseases includes infection, inflammation, topical medications, systemic medication, trauma, idiopathic etc. However, in some diseases like Acquired punctal stenosis in which the external opening of the lacrimal canaliculus is narrowed or occluded and Nasolacrimal Duct Obstruction in which there is tear duct blockage that results in obstruction of tear pathway, the etiology behind the disease remains unknown and is still under research. The main objective of this study was to determine if inflammation and infection would be the possible etiological cause behind these two diseases. In the present study, tear samples of 20 patients who were affected with NLD obstruction and APS disease were analysed. The presence of inflammatory marker MMP-9 was determined using InflammaDry point of care test in the tear samples of these patients and the results were positive indicating the presence of MMP-9. Further, the microorganisms present in the eyes of these patients were determined using Conjunctivitis Swab Test and were confirmed with Biochemical Tests. These include Staphylococcus aureus, Staphylococcus epidermidis, Moraxella catarrhalis, Serratiamarcescens. The amount of anti-oxidants and ascorbic acid present in these tear samples were also determined and it was found to be 19µM with a deviation of ±7µM in the case of ascorbic acid and 380µM with a deviation of ±150µM in the case of total anti-oxidant activity and they were correlated with normal values. The value of protein and LDH was found to be 3mg/mL and 160 U/L respectively. The presence of tear specific proteins such as Lactoferrin, Lysozyme, Albumin, Pre-Albumin, Protein-G were also determined using SDS-PAGE. The conclusion made from the result indicates that both Inflammation and Infection plays a role in the mechanism of these diseases.

I. INTRODUCTION

- The human eye is the organ that is responsible for our vision. It reacts to light and pressure and provides a three dimensional, moving image, normally coloured in daylight.
- Several diseases affect the eye directly or as a part of system wide problem. Of the 37 million people across the globe who are blind, over 15 million are from India. Over 75% of these are cases of avoidable blindness irrespective of age and gender. (Noopur Gupta *et al.*, 2013).
- The etiological cause for most of these diseases such as conjunctivitis, cataract, blepharitis have been determined and several methods of treatment have been administered.
- However, the etiological cause behind a few diseases that affect the eye and the possible solution to aid in curing them is still under research.

Nasolacrimal Duct Obstruction

- Tears normally drain through small openings in the corners of the upper and lower eyelids called puncta and enter the nose through the nasolacrimal duct.
- Tear duct obstruction prevents tears from draining through this system normally. If the tear duct is blocked, there will be backflow of tears and discharge from the eye.
- The most common cause is the failure of a membrane at the end of the tear duct (valve of Hasner) to open normally at or near the time of birth (Kushner BJ *et al.*, 1998).

Acquired Punctal Stenosis

- Acquired punctal stenosis is a condition in which the external opening of the lacrimal canaliculus is narrowed or occluded.
- This condition is a rare cause of symptomatic epiphora, but its incidence may be higher in patients with chronic blepharitis, in those treated with various topical medications, including antihypertensive agents, and especially in patients treated with taxanes for cancer *(*Kashkouli MB *et al.*, 2003*).*

II. AIM AND OBJECTIVES

AIM:

To identify the role of Inflammation and Infectious disease as an etiological cause in patients with obstruction of the tear drainage pathway.

OBJECTIVES

- To collect the tear samples from patients (35-60 years) in and around Chennai who are affected with NLD obstruction and Acquired Punctal stenosis.

- To determine the presence of Matrix Metalloproteinase -9(MMP-9), inflammatory marker in these tear samples.
- To determine the micro-organisms present if any, through Conjunctivitis Culture test/Swab test.
- To determine the total quantity of anti-oxidants and ascorbic acid content present in these tear samples
- To determine the amount of Protein and Lactate dehydrogenase in these tear samples
- To determine the level of Albumin, Pre-albumin, lysozyme, lactoferrin, Protein-G in these samples through SDS-PAGE.

STUDY DESIGN

- Patients (Age around 35-60 years) who were affected with NLD obstruction and Acquired Punctal stenosis will be chosen for this study.
- The samples from the patients will be collected from Ramana Eye Clinic, Alwarpet, Chennai and Regional Institute of Ophthalmology and Government Ophthalmic Hospital, Egmore.
- The patients who have no history or previous record of surgical operation with respect to the eye and who are not under the influence of eye-drops will be chosen for this study. For example, cataract surgery patients, LASIK surgery patients, etc will not be chosen.
- Twenty patients (Both Male and Female) will be taken for this study. They will be grouped according to the following table (Table 1) and will be tested for various parameters.

Table 1: Patient Grouping

Group Number	Type
Group1 (5 patients)	Patients with partial Acquired Punctal Stenosis
Group 2 (5 patients)	Patients with partial NLD obstruction.
Group 3 (5 patients)	Patients with complete Acquired Punctal Stenosis.
Group 4 (5 patients)	Patients with complete NLD obstruction.

III. METHODOLOGY

Step 1: Tear samples of Patients from all the 4 groups will be checked for the presence of MMP-9 using InflammaDry (Rapid Pathogen Screening Test) (Robert Sambursky *et al*., 2014).

Step 2: Presence of Micro-organisms in the tear samples of these patients belonging to each of these groups will be identified by performing Conjunctival culture test/ Swab test and they will be confirmed with the help of biochemical tests (Sharma *et al*., 2011) (Kavita *et al*., 2008).

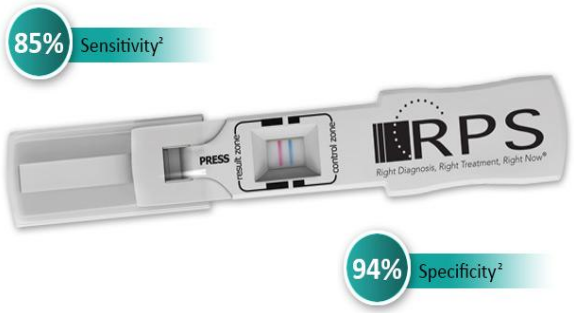
Step 3: 1 mL of tear samples from (Both right and left eye) will be collected with the help of capillary micropipettes and stored in Eppendorf vials. To each of these vials 0.2mL of extraction buffer will be added and stored at -20ºC until processed. The total quantity of anti-oxidants and ascorbic acid content present in these tear samples will be determined by FRASC assay and it will be correlated with the tears of healthy subjects (Choy *et al*., 2009).

Step 4: The protein and LDH content present in the tear samples of these patients will also be calculated by standards procedures and will be correlated with the tears of healthy subjects (Lowry *et al*., 1951) (King *et al*., 1965).

Step 5: The tear samples will also be subjected to SDS-PAGE analysis to determine the presence of protein fractions such as lysozyme, lactoferrin, PMFA (Proteins moving faster than albumin), Albumin, Protein- G (Annie Marie *et al*., 2010).

Step1: Sample Collection for InflammaDry Test. (Rapid Pathogen screening technology)

- ✓ 5µl – 10µl of tear sample will be collected from patients with clinical symptoms for Punctal stenosis and NLD obstruction with informed consent.
- ✓ These samples will be collected in the commercially available diagnostic kits from patients by experienced medical professional or lab technicians.
- ✓ The collected samples will be transported to the study site for further proceedings.



- InflammaDry® is the first and only, rapid, in-office test that detects MMP-9, an inflammatory marker. Using direct sampling microfiltration technology, InflammaDry® accurately identifies elevated levels of MMP-9 protein in tear fluid samples taken from the inside lining of the lower eyelid, the palpebral conjunctiva.
- InflammaDry® is a disposable, low cost test, that requires no additional equipment to administer or interpret results. Using four simple steps, InflammaDry® test results are achieved in just 10 minutes, aiding in the diagnosis of eye diseases before the patient leaves the office.

IV. RESULTS AND DISCUSSION

PRESENCE OF MMP-9 USING INFLAMMADRY TEST: GROUP – 1:

- The results of the inflammatory marker MMP-9in the tear sample of patients affected Partial Acquired Punctal Stenosis belonging to Group 1 was found. (Table 1.0 and Table 2.0)
- The MMP-9 level measured with the help of diagnostic kits appeared as follows,

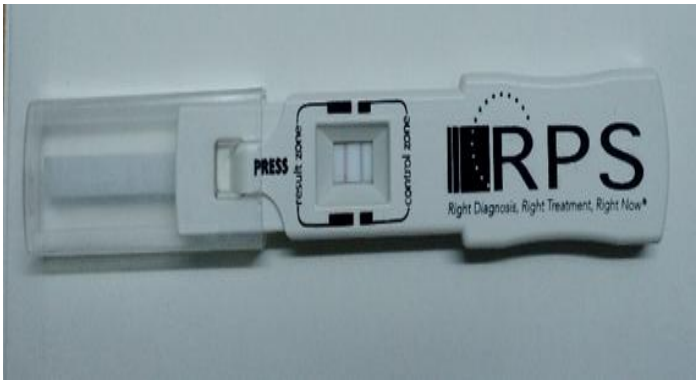


Fig 1: Presence of MMP-9 – High Level

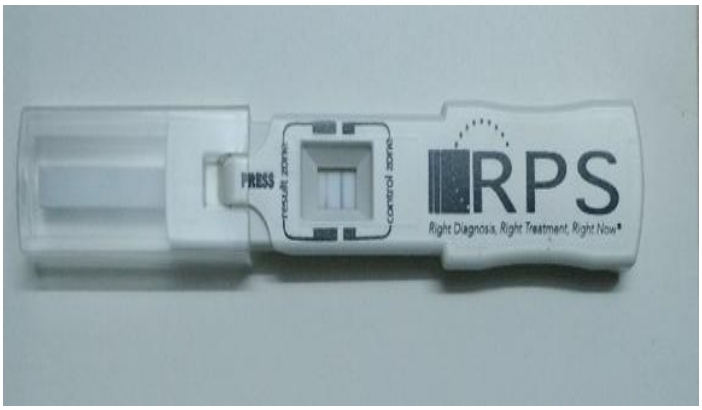


Fig 2: Presence of MMP-9 – Medium Level

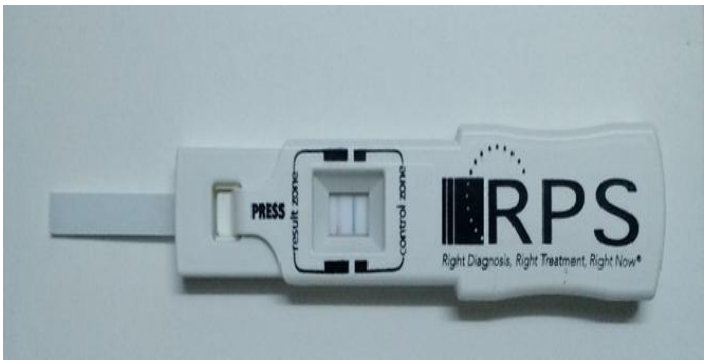


Fig 3: Presence of MMP-9 – Mild Level

Table 2: Presence of MMP-9 levels in the right eye of Patients with Partial Acquired Punctal Stenosis

Group 1 (Right Eye (OD)	Mild	Medium	High
Patient 1		✓	
Patient 2	✓		
Patient 3	✓		
Patient 4		✓	
Patient 5		✓	

Table 3: Presence of MMP-9 levels in the left eye of Patients with Partial Acquired Punctal Stenosis.

Group 1 (Left Eye (OS))	Mild	Medium	High
Patient 1			
Patient 2		✓	
Patient 3			✓
Patient 4			
Patient 5			

In Table 2.0 and 3.0, the results obtained for the levels of MMP-9 were recorded and it was found that in patients 1, 4 the right eye was affected and in patients 2,3,5 the left eye was affected with Partial Acquired Punctal Stenosis. This was also confirmed further by subjecting these patients to Slit Lamp Biomicroscopy Images of both the eyes (Fig 4).

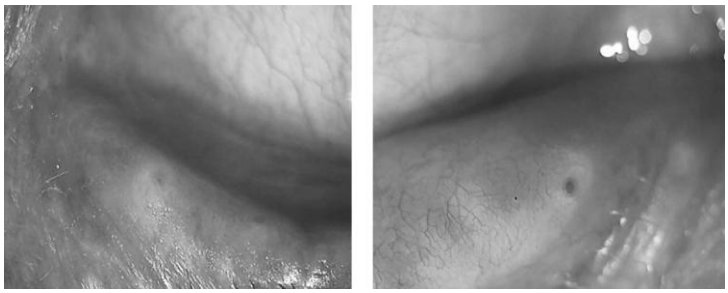


Fig:4. Less severe (Partial) Acquired lacrimal punctal stenosis (left) and normal punctal opening (right) are shown.

GROUP – 2:

The results of the inflammatory marker MMP-9in the tear sample of patients affected with Partial NLD obstruction belonging to Group 2 was found (Table 4.0 and 5.0).

Table 4: Presence of MMP-9 in right eye of patients with partial NLD Obstruction.

Group 2 (Right Eye (OD)	Mild	Medium	High
Patient 6		✓	
Patient 7	✓		
Patient 8			✓
Patient 9		✓	
Patient 10		✓	

Table 5: Presence of MMP-9 in left eye of patients with partial NLD Obstruction.

Group 2 (Left Eye (OS))	Mild	Medium	High
Patient 6	✓		
Patient 7			✓
Patient 8	✓		
Patient 9	✓		
Patient 10	✓		

From Table 4.0 and 5.0, it was found that in patient 7 the affected eye was the left eye, and in patients 6, 8, 9, 10 the affected eye was the right eye. These results also proved to be significant according to the results obtained by subjecting these patients to Slit Lamp Biomicroscopy Images of both the eyes.

GROUP 3:

The results of the inflammatory marker MMP-9in the tear sample of patients affected with complete Acquired Punctal Stenosis belonging to Group 3 was found (Table 6.0 and 7.0).

Table 6: Presence of MMP-9 in right eye of patients with complete Acquired Punctal Stenosis**.**

Group 3 (Right Eye (OD))	Mild	Medium	High
Patient 11	✓		
Patient 12			✓
Patient 13		✓	
Patient 14		✓	
Patient 15	✓		

Table 7: Presence of MMP-9 in left eye of patients with complete Acquired Punctal Stenosis.

Group 3 (Left Eye (OS)	Mild	Medium	High
Patient 11			✓
Patient 12		✓	
Patient 13			✓
Patient 14			✓
Patient 15			✓

From Table 6.0 and 7.0, it was found that in patients 11, 13, 14, 15 the left eye was affected with complete punctal stenosis and in-patient 12, this was observed in the right eye. These results again proved to be similar and was confirmed with the slit lamp biomicroscopy images. They can be understood with the help of the following image (Fig 5).

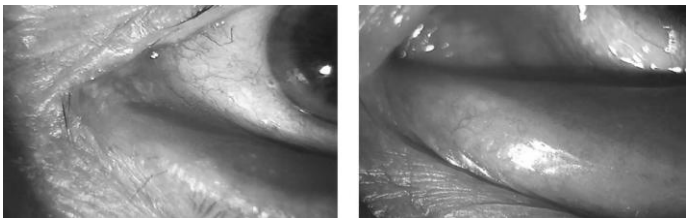


Fig:5. External lacrimal punctal opening is not visible (left) and very stenotic (right).

GROUP 4:

The results of the inflammatory marker MMP-9in the tear sample of patients affected with complete NLD obstruction belonging to Group 4 was found (Table 8.0 and 9.0).

Table 8: Presence of MMP-9 in right eye of patients with complete NLD obstruction.

Group 4 (Right Eye (OD))	Mild	Medium	High
Patient 16			✓
Patient 17		✓	
Patient 18		✓	
Patient 19	✓		
Patient 20			✓

Table 9: Presence of MMP-9 in left eye of patients with complete NLD Obstruction.

Group 3 (Left Eye (OS)	Mild	Medium	High
Patient 16		✓	
Patient 17	✓		
Patient 18			✓
Patient 19			✓
Patient 20		✓	

From Table 8.0 and 9.0, it was found that in patients 16, 17, 20, the right eye was affected with complete NLD obstruction and in patient 18 and 19 this was observed in the left eye. These results also proved to be similar and was confirmed with Slit Lamp Biomicroscopy images.

DISCUSSION

In the present study, the InflammaDry diagnostic test was carried out for all the 4 groups of patients. This test is designed to detect abnormally elevated MMP-9 present in the late phase of the inflammation cycle and therefore the results obtained maybe more clinically relevant than causal mechanisms or acute symptoms. In this study, it was found that the inflammatory marker MMP-9 is present in the tear samples of Patients affected with NLD obstruction and Acquired Punctal Stenosis. The level of MMP-9 had significant amounts of variation with respect to each group ranging between mild, medium and high. The presence of MMP-9 indicates that the quantity of MMP-9 is essentially ≥ 40ng/ml for these patients, which was similar to the result and studies carried out on patients affected with Dry Eye disease by Robert Sambursky *et al*., (2011).

Further increased activity of matrix metalloproteinases (MMPs), especially MMP-9, plays a critical role in wound healing and inflammation and is primarily responsible for the pathologic alterations to the ocular surface that leads to a dysfunctional tear state (Afonso *et al*., 1999). This correlates with the diseased condition of the patients taken for the study. The results obtained would thus help patients benefit from initial intervention with anti-inflammatory therapy.

AMOUNT OF ANTI-OXIDANT AND ASCORBIC ACID PRESENT IN TEARS:

The results obtained from the FRASC Assay for the determination of total anti-oxidant activity and ascorbic acid levels in tears of these patients were as follows,

Table:10. Amount of Anti-Oxidant Activity & Ascorbic Acid present among various Groups

Ascorbic Acid in normal tears: 30-40µM

Total Anti-oxidant activity in normal tears: 500-600µM (Camus Kar Man Choy *et al*., 2000)

Parameters	Group 1 (n = 5)	Group2 (n = 5)	Group3 (n = 5)	Group4 (n = 5)
Ascorbic acid (µM)	19±6.7	21±6.9	17±6.1	19±6.5

Total antioxidant Activity (µM)	367±149	386±157	352±145	371±151

n = number of subjects (patients taken for the study)
Values are mean ± SD.

DISCUSSION – ANTI-OXIDANT AND ASCORBIC ACID LEVEL**:**

The results obtained from this assay with respect to the total anti-oxidant and ascorbic acid content in the tear samples of patients with NLD and APS among the four groups were found to be similar to the work done by Camus Kar Man Choy *et al*., (2000). In addition, the data collected provides a baseline to support future clinical study to characterize the yet unidentified antioxidants in tears, and to investigate the effects of environmental conditions, antioxidant supplementation, aging, and ocular disease on the antioxidant status of human tears.

LEVEL OF PROTEIN AND LACTATE DEHYDROGENASE IN TEARS:

The level of protein in the tear sample of these patients determined by Lowry's method and King's method was found as:

Table 11: Amount of Protein & Lactate Dehydrogenase present among various Groups Protein in normal tears: 6-10 mg/mL LDH in normal tears: 200-250 U/L (Peter *et al*., 1982)

	Group 1 (n = 5)	**Group2 (n = 5)**	**Group3 (n = 5)**	**Group4 (n = 5)**
Protein (mg/mL)	**3.1±0.72**	**3.7±0.74**	**2.6±0.68**	**2.9±0.68**
Lactate Dehydrogenase (U/L)	**170**	**158**	**149**	**132**

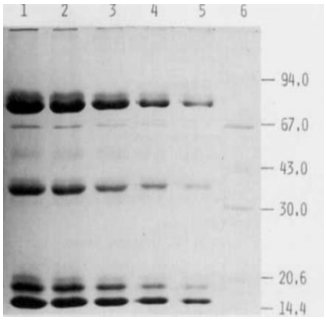
n = number of subjects (patients taken for the study)
Values are mean ± SD.

DISCUSSION:

In the present study, it was found that the amount of protein and lactate dehydrogenase in the tear samples of these patients was significantly lower when compared with the normal tear samples. This was similar to the work done by Andras Berta *et al*., (1999). The proteins present in tears play an important role in protecting the ocular surface and act as protective barriers against microorganisms. They also have a primary role in maintaining the tear film stability and hence protect the eye as a whole. The symptoms of these patients include excessive tearing, cyst formation and duct blockage. Hence, the low value of protein obtained in these tear samples correlates with their symptoms (Peter *et al*., 1982).

PROTEIN STUDIES BY SDS- PAGE:

- The 20 tear samples were electrophoretically separated in two runs of two gels each.
- Based on the molecular weight of protein standards, the fractions of protein present in the tear sample were separated and obtained as bands. In Fig 36, the protein molecular weight standards used were Lactoferrin (76,000), Albumin (62,000), two PMFA's (17,000 and 18,000), Protein-G (31,000) and lysozyme (15,000).



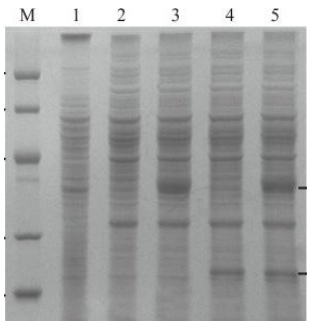


Fig: 6. SDS- PAGE of normal tear protein sample (left)
Fig: 7. SDS Page of Tear Sample – Diseased Patients (right)

DISCUSSION – SDS PAGE:

Based on Fig 7, it was found that level of all the molecular fractions of protein in the tear sample of these patients was significantly low when compared to the molecular fractions of protein obtained in tear sample of healthy subjects (Fig 6). These results were similar to the work done by Peter *et al*., (1982). The reason for the low level of these proteins lysozyme, lactoferrin, tear specific pre-albumin could be because they originate from the lacrimal gland. These patients tend to have damaged lacrimal gland. Also, the protein fractions, lysozyme and lactoferrin tend to have bactericidal properties. Since the level of these proteins are low, they are more easily prone to infections as well. However, in the case of Albumin and Protein-G their exact role has not yet been determined and is still under research.

SUMMARY AND CONCLUSION

A large number of people suffer from eye- related diseases on a daily basis. The etiological cause for most of these diseases has been identified and treatment methodologies have been administered. However, in the case of Acquired Punctal Stenosis and Nasolacrimal Duct Obstruction, the reason for the closing of the puncta of the eye or the blockage of the nasolacrimal duct which results in the obstruction of tears has not been determined and is still under research.

This study was performed to determine the etiological cause behind these two diseases. The Inflamma Dry test to determine the presence of Inflammatory marker MMP-9, in the tear sample of patients affected with both NLD and Acquired Punctal Stenosis showed positive results and the level of the inflammatory marker was ≥ 40 ng/mL, thereby indicating that inflammation could be a possible etiological cause.

Also, an analysis of anti-oxidants, ascorbic acid, proteins, lactoferrin, lyzozyme, tear specific pre-albumin, protein-G etc in the tear samples of these patients was carried out. The level of ascorbic acid in the tear samples of these patients was found to be 19µM for Group 1, 21µM for Group 2, 17µM for Group 3 and 19µM for Group 4 each of them with a standard deviation of ±7µM (approx.). The total anti-o oxidant activity present in the tear samples of these patients were 367µM for Group 1 patients, 386 for Group 2 patients, 352µM for Group 3 patients and 371µM for Group 4 patients each with the standard deviation of ±150µM (approx.). These were low when compared to the ascorbic acid and anti-oxidant content present in tears of healthy subjects, which is around 23µM

with a deviation of ±9.6 for ascorbic acid and 409µM with a deviation of±160 for total anti-oxidant activity. Also, the protein and LDH content present in the tear sample of these patients were found to be 3.1mg/mL and 170 U/L for Group 1, 3.7 mg/mL and 158 U/L for Group 2, 2.6mg/mL and 149 U/L for Group 3, 2.9mg/mL and 132 U/L for Group 4 patients. This was also low when compared with the protein and LDH content present in tears of healthy subjects which is around 7mg/mL for protein and 200-250 U/L for LDH.

The SDS-PAGE also revealed the presence of other fractions of protein such as Albumin, Tear specific Pre-Albumin, lysozyme, lactoferrin, Protein-G. It was found that the levels of all these protein fractions was significantly low when compared with the normal tear samples using Protein molecular standards.

Inflammation and infection were identified as potential etiological causes for these diseases. However, further research is required to treat these diseases and thereby administer effective treatment options.

SIGNIFICANCE OF THE STUDY

- Through this study we will be able to find out if inflammation and infection is present in patients with Acquired Punctal stenosis and NLD obstruction.
- If the result is positive with respect to inflammation in such patients then it could be correlated with other diseases, for example diseases like Conjunctivital chalasis with similar symptoms such as watery eyes also have shown to exhibit high levels of MMP- 9. These patients could thus benefit from initial intervention with anti-inflammatory therapy.
- If the result is negative, then one of the benefits is that it directs us away from inflammation. This is helpful because then we need not work on anti-inflammatories such as corticosteroids or cyclosporine to treat such patients. It also aids in the diagnosis of several ocular surface diseases.
- Thus, this study contributes to the further research in the treatment of such eye-related diseases and is helpful to devise a feasible treatment methodology for the same.

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