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RESEARCH ARTICLE

"TO STUDY THE ROLE OF TEAR FILM MATRIX METALLOPROTEINASE-9 (MMP-9) IN THE DIAGNOSIS AND MANAGEMENT OF INFLAMMATORY DRY EYE DISEASE USING POINT TO CARE RAPID MMP-9 IMMUNOASSAY KIT"

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Abstract

Purpose: To study the role of Matrix Metalloproteinase-9 (MMP-9) in the tear film for the diagnosis and management of inflammatory dry eye disease using point to care Rapid MMP-9 immunoassay kit.

Methods: This prospective interventional study was conducted at a tertiary eye care centre in a duration of two years. 300 eyes (Right Eye) of 300 patients with Dry Eye Disease (DED) were included in the study. After assessing the signs and grade of dry eye clinically, the patients were subjected to MMP-9 immunoassay of the tear film. The tear MMP-9 immunoassay results were interpreted using qualitative (positive or negative) and quantitative (reagent band density on a four-point scale: 0 = negative; 1 = weakly positive; 2 = moderately positive; 3 = strongly positive) methods. The patients who were tested negative for MMP-9 were treated with eye drop Carboxy methyl cellulose (CMC) 0.5% thrice a day, while those who were tested positive for MMP-9 were given eye drop Cyclosporine 0.05 % twice a day in addition to lubricants. Treatment and follow up was done for a period of six months and the response to treatment was re-assessed at the end of first week, first month, three months and six months.

Result: At presentation among 120 MMP-9 positive patients, 34%(41) were mild positive, 45%(54) were moderate positive, 21%(25) were severe positive. 88.98%(105) of 120, MMP-9 positive patients became mild positive and 10.8% converted to MMP-9 negative at the end of six months follow up. In MMP-9 positive group, mean reduction in OSDI from baseline 33.46 ±13.47 to 11.86 ±2.99, mean increase in Schirmer from baseline 9.22 ±1.64 to 13.15 ±2.42 and mean increase in TBUT from baseline 8.68 ±1.11 to 11.33 ±1.36 were observed at the end of six month follow up.

Conclusion: In this study, we found that there is a positive correlation between the MMP-9 immunoassay results with that of the severity of the signs and symptoms of DED. It was also observed that patients who were in MMP-9 positive group were found to have higher OSDI, lower Schirmer and TBUT values at baseline andthey showed significant improvement in the scores of dry eye with anti-

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inflammatory drops.Hence, topical anti-inflammatory eye drops plays a major role in the management of patients with severe DED.

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Introduction:-

Ocular surface plays a major role in maintaining the integrity of the corneal surface and providing a clear vision to the human eyes. Dry eye disease (DED) is a multifactorial disease of the ocular surface that results in symptoms of discomfort, visual disturbance and tear film instability that can cause damage to cornea. In-spite of high prevalence of DED, the discordance between symptoms, clinical signs, and diagnostic test results are inconsistent, making the diagnosis and treatment of this condition challenging[1].

DED has been classified as Evaporative dry eye (EDE), where tear hyperosmolarity is the result of an excessive evaporation of the tear film in presence of normal lacrimal function and the Aqueous-deficient dry eye (ADDE), where hyperosmolarity results from a reduced lacrimal secretion in the presence of a normal rate of tear evaporation[2].

Tear hyperosmolarity damages the ocular surface epithelium and sets off a cascade of inflammatory pathways which leads to tear film instability. MAP kinases, NFκB, IL-1, TNF-α, and MMP-9 are the major cytokines that are known to cause the inflammation on the ocular surface[3]. Matrix metalloproteinases are proteolytic enzymes that are released by stressed epithelial cells on the ocular surface and has consistently been shown to be elevated in the tears of patients with dry eyes. Increased MMP-9 activity in DED may contribute to a deranged corneal epithelial barrier function, increased corneal epithelial desquamation, and corneal surface irregularity[4]. Preformed MMP-9 may also be released from the secretory granules of neutrophils recruited by associated inflammation and sub-epithelial expression of MMP-9 parallels basement membrane degradation from human neutrophils[5].

DED induced ocular surface inflammation disrupts the epithelial and mucin layers, further exacerbating tear film breakdown. Suppression of inflammation creates a supportive environment for reversal of DED induced cellular changes[6]. Dry eyes symptoms as described by patients include burning, dryness, foreign-body sensation, ocular pain, blurred vision, photophobia, and visual fatigue. Clinical signs of dry eye include positive vital staining of ocular surface, decreased tear film breakup time and Schirmer's test value, reduced corneal sensitivity and decreased functional visual acuity[7].

At present, no single "Gold standard" sign or symptom that correlates perfectly with the DED has been established. Many biomarkers such as MMP-9, IL-1, IL-8, interferon gamma-induced protein (IP)-10, S100 calcium binding protein are being studied and provide insight into disease pathogenesis[8].

This discrepancy in the assessment of dry eye severity by clinicians and patients often results in patients receiving treatment with only artificial tears without consideration of the cause of the disease[9]. Confirmation of the presence of inflammatory dry eye by using inflammatory markers such as MMP-9 will drive the initiation of appropriate therapy. Hence, this study was conducted to estimate the levels of MMP-9 in the tear films of patients of DED and initiate the appropriate treatment based on subjective symptoms, clinical signs along with MMP-9 immunoassay assessment.Earlier tests which were available to measure the levels of MMP- 9 was complex which was difficult to quantitate. Western blotting techniques, immuno-histochemistry, enzyme leak immunoassays and MMP-9 activity measurements have been developed in recent times[10].

In our study we use inexpensive, disposable single use rapid detection immunoassay kit (InflammaDry) to measure tear MMP-9 which provides a result in minutes which can be conducted by trained paramedics in eye OPD itself. This new assay measures both active and latent MMP-9 (total MMP-9) in tears at a concentration > 40 ng/ml[11].

It appears that topical corticosteroids may be valuable in the management of inflammatory component of DED but their long-term use can cause ocular hypertension, cataract and opportunistic infections[12]. Cyclosporine is an immunomodulatory drug with anti-inflammatory properties, as well as having other properties relevant to manageDED. Cyclosporine is a fungal antimetabolite that inhibits IL-2 activation of lymphocytes Cyclosporine also

has anti-apoptotic effects known to reverse the normal epithelial cell/leukocyte relationship in dry eye disease, a result, not produced by corticosteroid treatment. Additionally, cyclosporine treatment has been reported to result in increased goblet cell density in the conjunctiva of subjects with dry eye disease[[13,14]. Hence we used cyclosporine as an anti-inflammatory intervention to treat inflammatory dry eye in our study at a tertiary care eye centre.

Materials and Methods:-

Study design, and setting :

This prospective, interventional study total 300 patients above the age of 18 years , with the symptoms of dry eye were included. The duration of the study was for a period of 2 years. After obtaining institutional ethical clearance, the clinical data and MMP-9 test was conducted in the right eye of all the 300 enrolled patient.Patients with history of ocular injury, contact lens use, ocular surgery, chronic inflammatory disease, those using topical or systemic immunosuppressive drugs were excluded from study.

After subjecting to the comprehensive ophthalmology evaluation, Ocular surface disease index (OSDI) questionnaire were given to all 300 patients. OSDI value > 14 is considered significant for detection of dry eye disease. Clinical diagnostic tests like Schirmer-I,FTBUT and tear MMP-9 immunoassay were conducted. Tear sample for MMP-9 immunoassay was taken from right eye lower fornix of patients. Based on tear MMP-9 levels , patients were divided in to two groups - MMP-9 positive and MMP-9 negative.

Intervention was given in form of carboy methyl cellulose 0.5% thrice a day in the MMP-9 negative patients. MMP-9 positive group received additional anti-inflammatory treatment in form of Cyclosporine 0.05 % twice a day dose and both group were followed up at first week, 4th week, 3rd month and 6th month after commencing the therapy.

Ocular Surface Disease Index

Detailed history of onset of DED and symptoms was taken based on Ocular surface disease index (OSDI). OSDI value > 14 is considered significant for detection of dry eye disease. The OSDI is a global assessment measure consisting of 12 questions, each scored by the patient. It has been used to evaluate the severity of symptoms and response to previous treatment in patients with dry eye. The OSDI scores range from 0 (no disability) to 100 (complete disability).

Fluorescein Tear Break-Up Time

The TBUT was evaluated 2 minutes after the infero-temporal bulbar conjunctiva is touched with a sodium fluorescein strip. Participants were instructed to blink, and the precorneal tear film would be examined under blue-light illumination with a biomicroscope and 10X magnification. The interval between the blink and the appearance of the first dark spot or discontinuity in the precorneal fluorescein-stained tear layer would be then recorded. TBUT < 10 is considered significant in dry eye patients (**FIGURE-1**).

Figure I:- Slit lamp imaging of Fluorescein tear break up time.

Schirmer Test

A Schirmer test (Schirmer I) was performed by placing Schirmer test strips (Watman filter paper no 41) over the lower eyelid margin, at the junction of the lateral and middle thirds, for 5 minutes without using anaesthesia. The strip wetting was measured and recorded in millimeters. Wetting of stip < 10 mm is considered significant to diagnose dry eye patients (**FIGURE-II**).

Figure II:- Procedure of doing Schirmer test.

Procedure Of Testing Matrix Metalloproteinase-9 In Tear Sample

MMP-9 Level in tear sample was tested using rapid detection immunoassay kit. It uses direct sampling microfiltration technology. Two antigen-specific antibodies capture MMP-9 antigens in the sample, and this complex is captured in a proprietary mode at the test result line, giving rise to a visually observable signal. The test requires 10 minutes for a result.

Figure III:-Collection of tear sample from lower fornix

Kit consists of 2 parts: a sterile sample collector and an immunoassay test strip in a plastic test cassette housing. After the sample collector is used to collect the tear fluid(**FIGURE-III**), it is assembled to the test cassette. Sample transfer to the test strip happens automatically without any pretreatment or dilution of the sample. The test is initiated when the absorbent pad of the test strip is dipped into a buffer solution(**FIGURE-IV**).

Figure IV:- Assembling Kit Parts(A), Dipping In Buffer Solution(B).

After 10 minutes, the result is visible in a readout window. The presence of 1 blue line (control line) indicates a negative (MMP-9, less than 40 ng/mL) result, whereas 2 lines (blue control line and red) confirms inflammation. Colour of redline can be compared for disease severity (Mild, moderate, severe). The tear MMP-9 immunoassay results were interpreted using qualitative (positive or negative) and quantitative (reagent band density on a four-point scale: 0 = negative; 1 = weakly positive; 2 = moderately positive; 3 = strongly positive) methods(**FIGURE-V**).

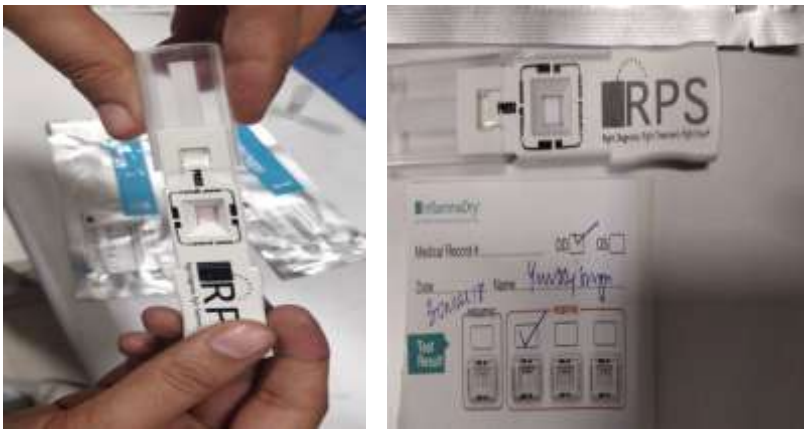

Figure V:- Test result interpretation (A,B).

Statistical analysis:

Categorical variables were presented in number and percentage (%) and continuous variables were presented as mean ±SD. Normality of data was tested by Kolmogorov-Smirnov test.Qualitative variables were compared using Chi-Square test and Quantitative variables were compared using Student t-test. Student's unpaired t-test was applied to test the difference between mean values in two groups. Paired t-test was used to see the change in a variables with respect to time.

Statistical package for social sciences (SPSS) version 18.0 was used for statistical analysis. A p value of <0.05 was considered statistically significant at 95% confidence level and Microsoft word and Excel have been used to generate graphs, tables etc.

Results:-

Demographics of the study populations:

A total of 300 Right Eye of 300 patients were included in the study with 37% males and 63% females. 95 patients were in the age group of 31-50 and 205 patients were in 51- 90 yrs with mean age of all the patients was 56.4 years.

Baseline Characterstics:

120 (40%) patients were found to be MMP-9 positive and 180 (60%) were MMP-9 negative. A total of 298 patients (99.3%) were followed up through the entire duration (6 months) of this study. 02 patients (1.6%) in MMP-9

positive group of 118 patients developed allergy to cyclosporine and were dropped from study. Among MMP-9 positive cases, 34%(41) were mild positive, 45%(54) were moderate positive, 21%(25) were severe positive.The demographic data of patients according to MMP-9 positivity and baseline tests values are shown in **Table 1.**

	MMP-9 positive	**MMP-9 negative**	**P Value**
No of patients	120	180	
Males: Female	49: 71	62:118	0.26
Mean BCVA (logMAR) With SD	0.67± 0.26	0.66± 0.27	0.93
BASELINE PARAMETERS			
Mean Age of Presentation With SD	56.87± 12.17	56.12± 11.57	0.048
Mean OSDI With SD	33.47± 13.47	12.50± 2.65	<.001
Mean SCHIRMER With SD	9.23± 1.64	12.41± 1.21	<.001
Mean TBUT With SD	8.68±1.11	11.48± 0.83	<.001

Table 1:- Baseline Demographic profile and Characteristics.

At presentation 95.8% (115) of MMP-9 positive group had OSDI > 14, while only 5%(09) of MMP-9 negative group had OSDI >14. 90%(108) of MMP-9 positive group had schirmer <10, while only 5% (09) of MMP-9 negative group schirmer <10. TBUT <10 was seen in 94.1%(113) of MMP-9 positive group and 6%(12) of MMP-9 negative group. The difference of values in both the groups at baseline were statistically significant (P<.001) **Table 2**.

BASELINE PARAMETER		**MMP-9 POSITIVE**			**MMP-9 NEGATIVE**
		MILD 41	MOD 54	SEVERE 25	180
OSDI	>14	115			9
	<14	5			171
SCHIRMER	<10	108			9
	>10	12			171
TBUT	<10	113			12
	>10	7			168

Table 2:- Baseline OSDI, Schirmer and TBUT in both groups.

At six month follow up only 11.6%(14) of MMP-9 positive group had OSDI > 14, while only 0.5%(1) of MMP-9 negative group had OSDI >14. 26.6%(32) of MMP-9 positive group had schirmer <10, while only 0.5%(1) of MMP-9 negative group schirmer <10. TBUT <10 was seen in 35%(42) of MMP-9 positive group and 0.5%(1)of

MMP-9 negative group. The difference of values in both the groups at baseline were statistically significant (P<.001).

In MMP-9 positive group, mean reduction in OSDI from baseline 33.46 ±13.47 to 11.86 ±2.99 (**CHART I**) , mean increase in Schirmer from baseline 9.22 ±1.64 to 13.15 ±2.42 (**CHART II**), mean increase in TBUT from baseline 8.68 ±1.11 to 11.33 ±1.36 (**CHART III**) seen at six month follow up. In MMP-9 negative group mean OSDI reduction from 12.5 ±2.66 to 7.83 ±1.98 seen. Mean increase in schirmer from 12.41 ±1.21 to 15.7 ±1.76, mean increase in TBUT 11.47 ±0.84 to 13.188 ±0.90 seen at six month.

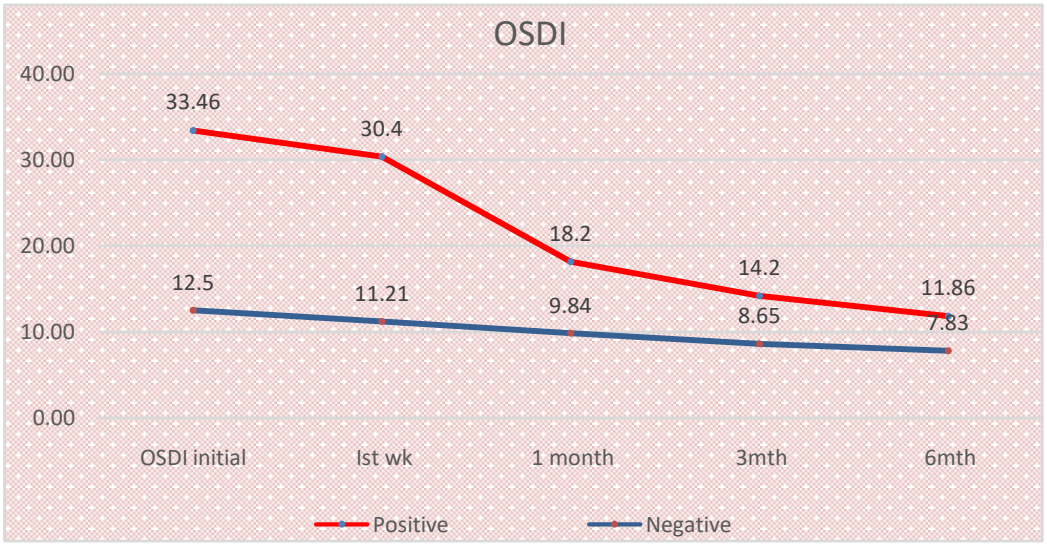

Chart 1:- OSDI Trends At Various Follow Up In Both The Groups.

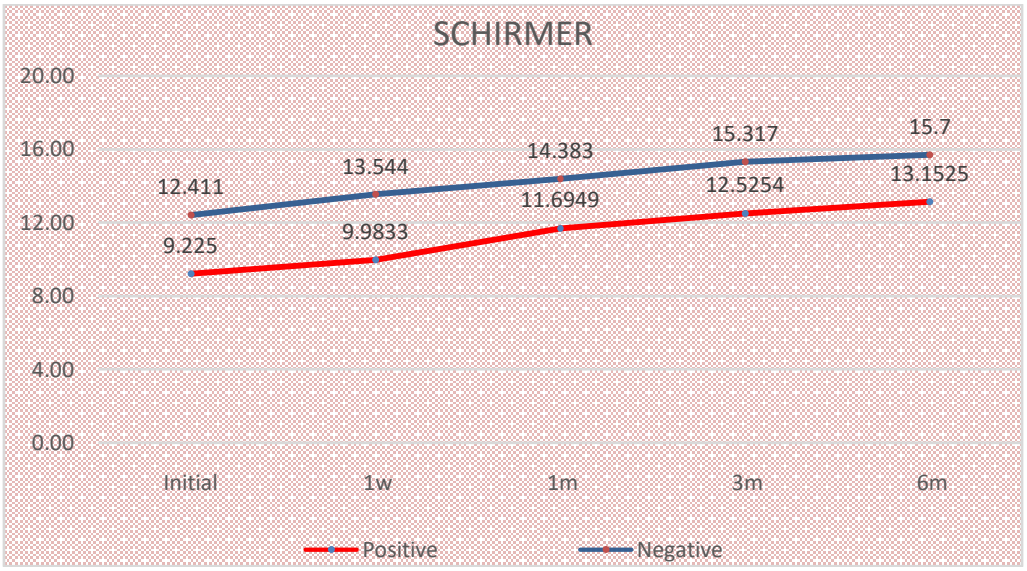

Chart 2:- Schirmer Trends At Various Follow Up In Both The Groups.

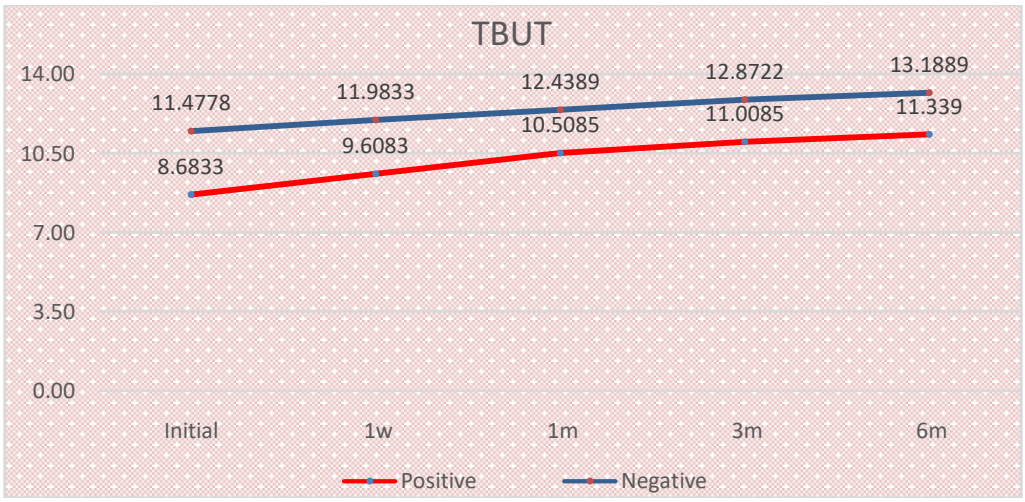

Chart 3:- TBUT Trends At Various Follow Up In Both The Groups.

Effect of cyclosporine 0.05 % in MMP-9 positive cases was the secondary outcome measure, which was assessed.After 06 month of treatment 88.98%(105) of 120, MMP-9 positive patients were mild positive and 10.8% converted to MMP-9 negative. 02 patients (2.3%) in MMP-9 positive group of 120 patients developed developed redness, itching, burning sensation at one week follow up to cyclosporine and were dropped from study.

There was a statistically significant treatment outcome with respect to the mean reduction in OSDI, Schirmer and TBUT ($p < 0.001$) along with change in MMP-9 status with treatment in both the groups.

Discussion:-

Dry eye disease is a highly disabling condition with multifactorial etiology that results in symptoms of discomfort, visual disturbance and tear film instability with potential damage to the ocular surface.The prevalence of DED is variable and ranges from 5 to 50%. Prevalence increases with age and signs shows greater increase per decade than symptoms. Women have a higher prevalence of DED than men and this differences become significant with age. The economic burden and impact of DED on vision, quality of life, work productivity, psychological and physical impact of pain are considerable[15].

Risk factors of DED are categorised as modifiable or non-modifable and as consistent, probable or inconclusive. Asian ethnicity is a consistent risk factor. Risk factors for dry eyes also include patients taking systemic medications (antihypertensives, antidepressants or hormone replacement therapies) patients with autoimmune inflammatory diseases, contact lens wearers, laser in-situ keratomileusis (LASIK) and refractive surgery patients, and postmenopausal women[16].

Human tear film is not a homogenous structure and it is classically divided into three distinguished layers. Innermost Mucin layer is 2.5 to 5 µm thick residing directly at the surface of cornea and form a gel-like structure providing an easily wettable surface. Middle Aqueous layer is of approximately 4 µm thick. It contains numerous water soluble and insoluble components such as electrolytes, proteins, peptides and small molecule metabolites, tear lipocalin and lysozyme. Outermost, Lipid layer is a relatively thin, 0.015 to 0.160 µm thick layer[17,18].

The TFOS DEWS-II Tear Film Subcommittee has recommended a two-phase model of the tear film, which has a lipid layer overlying a muco-aqueous phase. Non-polar lipids make the majority of the tear lipid layer spreading onto the muco-aqueous layer by an underlying layer of polar lipids [19].Delphi Dry Eye Panel Report in 2006, proposed that inflammatory mechanisms are involved in the pathophysiology of dysfunctional tear syndrome. Changes in tear composition in dysfunctional tear syndrome may destabilize the tear film and cause ocular surface epithelial disease[20].

Numerous extrinsic and intrinsic factors can trigger DED by impacting tear film stability by activating osmotic or mechanical stress mechanisms. This leads to apoptosis, ocular cell damage, and release of inflammatory mediators,

increasing ocular surface stress and leading to potential epithelial damage [21]. MMPs play a vital role in wound healing and inflammation. MMP-9 has been found to be of central importance in cleaving epithelial basement membrane components and tight junction proteins that maintain corneal epithelial barrier function MMP-9 belongs to the gelatinase group of metalloproteinases that degrade denatured collagen, native collagens type IV, V, and VII and elastin. Expression of MMP-9 by the ocular surface epithelia in normal healthy eyes is low. Increased MMP-9 activity has been associated with disruption of corneal epithelial barrier function and corneal surface irregularity in an experimental murine model of dry eye[22].

It is well established that perceived symptom severity may not equate to clinical signs of disease, and there exists a significant proportion of patients who have conflicting signs and symptoms.Also, symptoms and signs can vary greatly depending on the environmental conditions.

The most common routinely used objective diagnostic test for dry eye includes Schirmer test, Tear break up time (TBUT) and Fluorescein staining of ocular surface. The reported sensitivity and specificity of routine dry eye diagnostic methods, such as the Schirmer test, TBUT and OSDI, show a variable sensitivity and specificity.

The Ocular Surface Disease Index (OSDI) is a questionnaire that was developed to identify and quantify the common symptoms associated with DED. This assessment has been found to be subjective, lack specificity, and prone to operator-dependent analytical errors, preventing it from routine clinical use.

Schirmer tear has been in use for decades, lacks standardization and is inaccurate and unrepeatable because of the reflex secretion produced by its irritating nature. However, the low cost of strips and ease of application has led the Schirmer tear test to become the most common clinical test for lacrimal secretary function in dry eye. TBUT is considered to be more reliable than the Schirmer test, because it is repeatable and minimally invasive, however, the instillation of a topical anesthetic can destabilize the tear film and lead to an artificially accelerated TBUT. Ocular surface staining with vital dyes has been used to diagnose dry eye disease. The disadvantage of staining is that dry eye cannot be clinically differentiated from other conditions that can alsolead to ocular surface staining such as topical medication toxicity, poor lid apposition, underlying infection or trauma.

Diagnosis of this multifactorial disease may be improved with a protocol inclusive of multiple diagnostic tools. Elevated MMP-9 levels in patients with moderate to severe dry eye disease correlate with clinical examination findings. A noninvasive, inexpensive, disposable test that can accurately aid in the confirmation of the diagnosis of inflammatorydry eye may provide valuable information without imposing infrastructure challenges. Using this rapid commercially available immunoassay which detects elevated levels of MMP-9 (40 ng/mL) in tears to confirm the diagnosis of dry eye disease can be very useful for treating ophthalmologists.

Chotikavanich et al evaluated production and activity of metalloproteinase (MMP-9) on the ocular surface in patients with dysfunctional tear syndrome (DTS) and determined correlation between MMP-9 activity and clinical parameters. Tear MMP-9 activities showed significant correlation with symptom severity scores, fluorescein tear break-up time, corneal and conjunctival fluorescein staining. Tear MMP-9 activity was significantly higher in patients with DTS. MMP-9 appears to be a potentially useful biomarker for diagnosing, classifying, and starting anti inflammatory therapy.

Sambursky et al in his prospective, sequential, masked, multi-centre clinical trial, performed InflammaDry, ready to use MMP-9 Immunoassay tests on 206 patients. 143 patients were with clinical signs and symptoms of dry eyes and 63 healthy individuals as controls. Participants were assessed as healthy controls or for a clinical diagnosis of dry eye using the Ocular Surface Disease Index, Schirmer tear test, tear breakup time, and kerato-conjunctival staining. The sensitivity and specificity of MMP-9 Immunoassay was compared with clinical assessment. InflammaDry, MMP-9 Immunoassay showed sensitivity of 85% (in 121 of 143 patients), specificity of 94% (59 of 63). He concluded that when compared with the clinical assessment, Both findings were statistically significant ($p < 0.001$).

Kaufman et al evaluated the importance and practicality of testing for matrix metalloproteinase 9 (MMP-9) in dry eye and ocular surface disease using Enzyme-linked immunosorbent assay, a rapid immunoassay. He found MMP-9 measurement is sensitive and accurate for diagnosing dry eye and ocular surface disease and compares favourably in

both sensitivity and specificity against the existing methods of dry eye diagnosis. The presence of elevated MMP-9 on the ocular surface will identify those patients who should receive anti inflammatory therapy, such as cyclosporine, and may predict those patients who will respond to this therapy.

Brignole et al showed that 6 months of treatment with topical cyclosporine 0.05% can reduce inflammatory markers in patients with moderate to severe dry eyes with cyclosporine ophthalmic emulsion.

This study was aimed to detect tear matrix metalloproteinase-9 (MMP-9) using ready to use point to care MMP-9 Immunoassay kit in Eye OPD to diagnose inflammatory dry eye diseases and compare results with traditional dry eye tests and managing it effectively using Anti-inflammatory drugs. In this study we observed that MMP-9 positive group was associated with significant high OSDI, significant low Schirmer and TBUT. Improvement in the scores of OSDI, Schirmer and TBUT were observed with anti-inflammatory treatment in MMP-9 positive group. There was a significant reduction in MMP-9 severity at each follow up.1.6% patients in MMP-9 positive group developed ocular side effects from topical cyclosporine 0.05% in form of increase redness, pain, irritation in eyes. None in MMP-9 negative developed any side effects from medication.

This study found that tear MMP-9 detection at initial presentation along with other standard available tests in form of OSDI, Schirmer and TBUT can be of diagnostic importance in dry eye and its detection and measurement may be a reliable indicator of those patients who will respond to anti-inflammatory therapy.Availability of this practical method to detect elevated levels of MMP-9 in eye OPD may facilitate the targeted therapeutic management of symptomatic patients at early stage.

Conclusion:-

This study found tear sample MMP-9 detection using rapid immunoassaykit along with standard old diagnostic tests correlates well with the disease severity. MMP-9 test is indicated to detect hidden cases of inflammatory dry eye disease that may not be easily identified through the clinical examination because inflammation is often present long before clinical signs appear.Correctly diagnosing inflammatory dry eye will help in early initiation of effective targeted therapeutic treatment.

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Nil.

Conflicts of interest:
There are no conflicts of interest.

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