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TO ASSESS THE ROLE OF TEAR-FILM BIOMARKERS IN THE DIAGNOSIS AND MANAGEMENT OF THE DRY EYE DISEASE.

Ophthalmology

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ABSTRACT

Aims: To assess the level of different tear film biomarkers & their association in dry eye diseases.
To assess role of Omega 3, Omega 6 supplements as therapeutic role among dry eye disease patients.
Settings and Design: Prospective, Non-Randomized and Observational Study
Materials and Methods: 42 patients that met the inclusion and exclusion criteria were enrolled into the study at the Department of Ophthalmology, Dhiraj Hospital Piparia, Waghodia, Vadodara, after getting approval from Ethics committee.
All patients undergone detailed ophthalmological examinations consisting of History, Visual acuity (both uncorrected and Best corrected visual acuity) anterior segment examination using slit lamp bio microscopy tear film evaluation both for severity of dry eyes and biomarkers, the role of omega3 and omega6 fatty acids supplement was correlated with tear film bio markers of our study subjects.
Results:

- Out of 42 patients 19.07% people were having severe dry eyes and 38.09% were catagorised as moderate and 42.87% as mild dry eye disease.
- The levels of cytokines IL1-alpha,IL-6, and Tnf-alpha was significantly high in patients with dry eye disease while MMP-9 level was not significant.
- Correlation of combination therepy with omega 3& omega6 fatty acids supplements found beneficial in study group compared to control group and was statistically significant among study population. ($P<0.002$)

Conclusion: This study helped in identifying the role of nutrition and tear film biomarkers and nutritional component of dry eye disease. The study concluded that the role of nutritional supplements decreases the amount of inflammatory cytokines in tear film, but has no role in increasing tear film production as well symptomatic relief for patients with dry eye disease.

KEYWORDS

Dry eye disease, Tear film biomarkers, Omega3 and omega 6 nutritional supplement.

INTRODUCTION

Dry eye disease (DED) is a multifactorial inflammatory disorder that is characterised by ocular discomfort, visual disturbances, tear film instability, increased tear osmolarity and inflammation[1,2]. It can be primarily classified into several major subtypes, including aqueous-deficient dry eye (ADDE) and evaporative dry eye. In patients with evaporative dry eye, lipid-deficient dry eye (LDDE) is the most common cause. the most common cause of LDDE is meibomian gland dysfunction (MGD)[3].

For aqueous-deficient dry eye (ADDE), a reduced lacrimal secretion causes the high osmolarity gradient. When the ocular surface epithelia is exposed to such an oxidative stress, intracellular signaling pathways are triggered, involving inflammatory mediators[4,5]. These mediators initiate the synthesis and release of pro-inflammatory cytokines, chemokines, and matrix metalloproteinases (MMPs)[6,7].

A biomarker, as defined by the National Institute of Health (NIH), is "A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to therapeutic intervention" [8]. Goblet cell loss is another important factor to considering the pathogenesis of DED, triggered by the large amount of pro-inflammatory cytokines on the ocular surface. Loss of goblet cells decreases the amount of mucus present on the ocular surface, worsening the hyperosmolarity. It is also said to lower the immune tolerance in mucosal tissues[9].

A biomarker is measured objectively and often represents the pathogenic and biological responses in the body. The use of a biomarker will lead to better diagnosis, categorization, and more effective management of DED[10,11].

The Role of OMEGA 3 & OMEGA 6 Fatty Acids in management of dry eye disease is controversial Aims of our study is to assess OMEGA 3 & OMEGA 6 Fatty acids beneficial role fordry eye diseases patients.

METHODS AND MATERIAL

Study setting

The study was conducted in Department of Ophthalmology, Dhiraj Hospital, SBKS Medical Institute and Research Centre, Piparia, Vadodara from January 2020 to April 2020.

Study design: observational study, Prospective study.
Sample size: 60 geriatric patients
Patient selection was random

Study population:
Total 60 geriatric age group of patients that visited Ophthalmology Department, Dhiraj hospital from March 2019 toApril 2020 were enrolled in the study.

Study selection Criteria:
INCLUSION CRITERIA:

- Age of patients >55 years
- Subjects with dry eye disease.
- Based on OSDI score subjects with presumptive signs of dry eyes.
- Willing to participate in study.

EXCLUSION CRITERIA:

- History of regular artificial tears usage.
- Self-reported dry eye symptoms over the past 6 months.
- Active ocular infection.
- Active allergic conjunctivitis.
- Use of any medication indicated to increase tear production.
- Sporadic use of any medication that could contribute to risk of dry eye,

such as beta blockers, diuretics, or systemic anticholinergic medications. (stable use for at least one month before study was permitted.)

Method Of Data Collection:

Patients of both gender (male, female) and age 55 years that visited Department of Ophthalmology were screened for the study with the aim to evaluated for tear film biomarkers in patients of dry eye disease .All the eligible study subjects were explained Patient Information sheet (PIS) in detail by the Principal Investigator (PI) in the language comfortable to the patient and sufficient time provided to the patient for making the decision to participate in the study. After patient's oral consent for the participation the study procedure was explained in a detailed fashion by the PI thereby taking the subject's signature on Informed Consent Form to confirm his participation in the study. After informed consent inclusion- exclusion criteria were reviewed and the following assessment was carried out.

Assessment of the patient:

A detailed ocular and medical history of the enrolled patient was taken followed by complete eye examination.

- Visual parameters in accordance with the Study Proforma were recorded; Uncorrected Visual Acuity (UCVA), Best Corrected Visual Acuity (BCVA)
- using Snellen's Visual Acuity Chart.
- Complete anterior segment examination was done using slit lamp bio microscope with special attention to the tear film evaluation.
- The collected tear film samples were sent for analysis to department of biochemistry, Dhiraj hospital SBKS MI&RC, For assessment of tear film biomarkers.

The following tests were performed on all patients:

Tests for Dry eye severity:

Schirmer's Test I:

The tear strip was folded at the 0 mm mark and placed at the junction of the medial two thirds and lateral one thirds of the lower eyelids and allowed to stay in place for 5 minutes. A value of less than 15 mm in 5 minutes was taken as abnormal."

Schirmer's Test II:

Topical anaesthesia by paracaine (0.5%) eyedrops was instilled in both eyes. Excess drops were wiped off from the inferior fornix. Tear strips were put for 5 minutes. A value of less than 15 mm in 5 minutes was considered abnormal.

Tear Film Break Up Time(TBUT):

A Fluorescein strip after wetting with a drop of sterile saline, was applied in the inferior conjunctival cul-de-sac without the use of topical anesthesia. The patients was asked to blink for 3-4 times.Thepatient was asked to open their eyes and refrain from blinking while performing the test. The tear film was then examined using a broad beam of the slit lamp with the blue cobalt filtered light. The time lapse between the last blink to the appearance of the first random dry spot was taken as the tear film break up time. A value of less than 10 seconds was taken as abnormal."

The above tests were conducted at room temperature with fans switched off, and all readings were taken by a single observer.

Tear film function including tear film stability (tear film break-up time, TBUT) and tear secretion (Schirmer's I test, schirmer's Il) and Slit lamp examination of the tear meniscus height was evaluated.

Ocular surface disease index scoring system:

Ocular Surface Disease Index© (OSDI©)2

Patients were asked the following 12 questions, and circle the number in the box that best represents each answer. Then, boxes were categorised as A, B, C, D, and E according to the instructions beside each.

Have you experienced any of the following *during the last week?*	All of the time	Most of the time	Half of the time	Some of the time	None of the time
1. Eyes that are sensitive to light? . .	4	3	2	1	0
2.Eyesthatfeelgritty?...	4	3	2	1	0
3. Painful or sore eyes?	4	3	2	1	0
4. Blurred vision?	4	3	2	1	0
5. Poor vision?	4	3	2	1	0

Sub total score for answers 1 to 5 (A)

Have problems with your eyes limited you in performing any of the following *during the last week?*	**All of the time**	**Most of the time**	**Half of the time**	**Some of the time**	**None of the time**	**N/A**
6.Reading?.....	4	3	2	1	0	N/A
7. Driving at night?	4	3	2	1	0	N/A
8. Working with a computer or bank machine (ATM)?. . .	4	3	2	1	0	N/A
9.WatchingTV?	4	3	2	1	0	N/A

Sub total score for answers 6 to 9 (B)

Have your eyes felt uncomfor table in any of the following situations *during the last week?*	**All of the time**	**Most of the time**	**Half of the time**	**Some of the time**	**None of the time**	**N/A**
10.Windycondit ions?.....	4	3	2	1	0	N/A
11. Places or areas with low humidity (very dry)?	4	3	2	1	0	N/A
12. Areas that are air conditioned?. . .	4	3	2	1	0	N/A

Sub total score for answers 10 to 12 (C)

Add subtotals A, B, and C to obtain D (D=sum of scores for all questions answered) **(D)**

Total number of questions answered (do not include questions answered N/A) **(E)**

Please turn over the questionnaire to calculate the patient's final OSDI© score.

Evaluating the OSDI© Score[1]

The OSDI© was assessed on a scale of 0 to 100, with higher scores representing greater disability. The index demonstrates sensitivity and specificity in distinguishing between normal subjects and patients with dry eye disease. The OSDI© is a valid and reliable instrument for measuring dry eye disease (normal, mild to moderate, and severe) and effect on vision-related function.patients categorized into mild, moderate and severe dry eye disease on the basis of these tests.

Molecular tests for assessing tear film biomarkers:

Tear film samples collected from both the eyes using current methods for tear sampling include: collection from Schirmer strips; various types of collectors, such as sponges or rods positioned in the conjunctival meniscus to be impregnated by tears; and glass capillaries or micropipettes with a disposable, sterile mini-tip at the outer conjunctival cantus micro capillaries.

Although Schirmer strips are comfortable for the patients, the retention of some proteins with clinical significance, such as serum-derived proteins (e.g. albumin) and those with molecular weight <40 kDa (e.g. lysozyme), is the main limitation in tear protein analysis.[19–21,25] Because of the lower impact on tear protein profiles, tear collection using glass capillaries is considered the most appropriate for tear protein analysis, Schirmer strips being recommended only for analysis of multiple cytokines.[1]

Samples were collected by shirmer strip as it is most compliant method for sample collection and my study was mainly stressed on cytokine analysis of tear film biomarkers.

Collected samples sent to biochemistry laboratory of Dhiraj hospital

for analysis of tear film biomarkers, Several inflammatory cytokines/chemokines (such as IL-1, IL-6, TNF-α, metalloproteinase (MMP)-9, have been found to be significantly increased in tears from DED patients. In our study we mainly analysed the IL-1,IL-6,TNf-a, and MMP and compared with different types of severity of dry eye disease and their association with underlying diseases.

RESULTS AND STASTICAL ANALYSIS:

Out of total 60 geriatric patients, total 42 were enrolled in study as 6 patients reported as active ocular infection in form of viral conjunctivitis,10 patients were using anti-hypertensive medications such as beta-blockers and 2 were not willing to participate in the study.

All patients were categorised in three groups:

A) severity of dry eye on the basis of tear film evaluation
B) Levels of tear film biomarkers in the tear film
C) Response with combination therapy of omega3 and omega 6 fatty acids supplementation.

A. Severity of dry eyes:

On the basis of schirmer's I and II ,TBUT, TMH and OSDI score the following result obtained and patients categorised as mild, moderate and severe dry eyes. Out of 42 patients enrolled 8 patients reported symptoms and signs of severe dry eyes, 16 patients categorised with moderate dry eye disease and 18 patients had only symptoms and sign of mild eye disease. The distribution of this group was independent of variables like systemic diseases, occupation, indoor work, outdoor work, unlike other studies.

Out of 42 patients 19.07% people were having severe dry eyes and 38.09% were catagorised as moderate and 42.87% as mild dry eye disease.

B. Assessment of Tear film Biomarkers

All the tear film specimens collected are sent to biochemistry laboratory of Dhiraj hospital for analysis of tear film biomarkers. Among all the biomarkers focus of study was mainly on assessment of Il-1beta,IL-6, TNF-alpha and MMP-9, and was mainly compared with association of inflammatory and non-inflammatory ocular diseases.

Table 1

Comparison of tear film biomarkers (Il-1,IL-6,TNF-alpha,and MMP-9) between dry eye disease with or without systemic inflammatory disease

Cytokine	Use in DED without systemic inflammatory disease	Use in DED with systemic inflammatory disease
IL-6	Raised in DED compared to controls, and correlates well with Schirmer's test, TBUT, and OSDI score	Raised and correlated well with ocular surface parameters in SS patients
IL-1β	Raised in DED compared to controls	Raised and correlates well with ocular surface parameters in ocular GVHD and SS patients
TNF-α	Raised in DED compared to controls, correlates well with Schirmer's test and OSDI scores	Raised and correlated with Schirmer's test score in SS patients but decreased in SJS patient
MMP-9	Variable	Findings supported by few literature
*IL=Interleukins, *MMP=metrix metalloproteinase (degradative enzyme),*Tnf=tumour necrosis factor.	*DED=Dry eye disease,*TBUT=Tear film break up time.	*SS=Sjogren's syndrome,*GVHD=Graft versus host disease,*SJS=Stevens jhonson syndrome.

Pro-inflammatory cytokines

Interleukin-6

Due to DED having an inflammatory origin, pro-inflammatory cytokines are well known for being possible biomarkers in DED. Among which, interleukin-6 (IL-6) has been supported as the best probable indicator for the disease. This study has revealed that IL-6 levels correlate well with ocular surface parameters, notably a negative relationship with Schirmer's test score and tear film break-up time (TBUT),[2,8,19,24,27] and a positive relationship with the ocular surface disease index (OSDI) score.[4,18,21] Moreover, some studies have found that treatment of dry eye with both artificial tears, steroids, and warm compression would concomitantly lower the level of IL-6.[1,5,25]

Tumor necrosis factor-alpha

Tumor necrosis factor alpha (TNF-α) is another pro-inflammatory cytokine that can stimulate MMP activation and limit fibrosis.[10,28] It could reduce tear production.[21] Multiple studies have pointed out its correlation with disease severity, being raised significantly in DED patients,[4,21,24,27] and having correlations with OSDI score and Schirmer's test score.[8,18,21]

Interleukin-1 beta

The pro-inflammatory cytokine IL-1β is an important mediator of inflammation. While a number of studies have revealed a difference in IL-1β levels between patient and control populations,[17,21,26] some other studies did not find any significant relationship between its levels and disease severity.[4,19,22,27] However, administration of dry eye treatment was found to significantly decrease tear IL-1β levels.[1,5]

Matrix metalloproteinase-9

While MMP-9 has a significant role in DED without an underlying cause, it has limited potential in DED due to an underlying cause. This may show that MMP-9 does not have a significant role in most systemic inflammatory diseases.

No significant differences of cytokines and MMPs extracted from clinical Schirmer strips by comparison of OD versus OS groups. All data are expressed as mean ± SE, $n = 42$ for all groups, except TNF-α, for which $n = 27$. OD (*black bars*) and OS (*gray bars*) were compared by one-way ANOVA Tukey–Kramer test and the value of $P < 0.05$ was determined to be significantly different.

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FACTORS	OSDI		TBUT		SHIRMER'S I&II	
	P	P	P	P	P	P
IL-1alpha	0.128	0.302	0.091	0.480	−0.826	<0.001
IL-6	0.106	0.442	-0.111	0.312	−0.644	<0.001
TNF-alpha	0.006	0.966	0.102	0.420	-0.414	0.020
MMP-9	-0.122	0.401	0.061	0.662	-0.582	<0.001

C. Role of supplements:

In our study we found that role of combination therapy with both omega3 and omega 6 fatty acids is associated with decrease in inflammatory cytokines and improvement in tear film stability after treatment.

In our study out of 42 patients 21 was observed as control group who was not provided with placebo treatment and rest was given supplementation of both omega3 and omega 6 fatty acid in the form of tablets and was studied for one year. There was reduction of inflammatory mediators like IL-1alpha, and IL-6 whereas Tnf-alpha and MMP-6 was variable among both control and study groups.

DISCUSSION:

Multiple trials looked at the effect of oral supplement of omega-3 and omega-6 in the treatment of DES. A large cross sectional study by Miljanović et al.20 has suggested that a higher dietary intake of omega-3 fatty acids is associated with a decreased presence of DES in geriatric patients.

Barabino et al.21 evaluated the effect of linoleic acid and gammalinolenic acid, on chronic ocular inflammation in 26 patients with aqueous-deficient keratoconjunctivitis sicca. Statistically significant changes in symptoms ($P < 0.005$), lissamine green staining ($P < 0.005$), and ocular surface inflammation ($P < 0.05$) occurred in the study group compared with controls.

Aragona et al.22 evaluated the effect of oral omega-6 essential fatty acids on PGE(1) tear content and signs and symptoms of ocular discomfort in patients with Sjögren's syndrome(SS). This was a randomized, double-masked, controlled, clinical trial involving 40 patients with primary SS. Twenty patients received omega-6 supplements while controls received placebo. In omga-6 treatment group significant changes were noted in the following: increased tear PGE1 levels, reduction of DES symptom score, and improved corneal fluorescein stain.

Although much of the evidence was uncertain, long-chain omega-3 supplements may have little to no benefit, relative to placebo, on dry eye symptoms, but did improve some clinical signs. There was a beneficial effect on dry eye symptoms when omega-3 supplements were combined with standard dry eye treatments (eg, artificial tears, eyelid warm compresses, corticosteroid eye drops) compared to standard treatment alone, and when long-chain omega-3 supplements were compared with omega-6 supplements. The most common side effect was temporary gastrointestinal problems.

For combined omega-3 and omega-6 supplements, relative to placebo, there was no benefit for tear production, and a small amount of improvement in the stability of the tears. Effects on other clinical measures, including dry eye symptoms and side effects, could not be clearly determined. It is also unclear whether other types of supplement combinations are effective for treating dry eye. We have low to moderate confidence in the evidence for all outcomes.

These findings suggest that long-chain omega-3 and omega-6 supplements may have a role in managing dry eye, however the evidence is currently inconsistent and more research is needed.

This study helped in identifying the role of nutrition and tear filmbiomarkers and nutritional component of dry eye disease. The study concluded that the role of nutritional supplements decreases the amount of inflammatory cytokines in tear film, but has no role in increasing tear film production as well symptomatic relief for patients with dry eye disease.

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