EFFECT OF TOPICAL ANTI-GLAUCOMA MEDICATIONS ON OCULAR SURFACE: A PROSPECTIVE COHORT STUDY

Thesis

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Ophthalmology

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DECLARATION

I hereby declare that this project titled "**Effect of topical anti-glaucoma medications on ocular surface: a prospective cohort study**" is the bonafide record of my original research. It has not been submitted to any other institution for the award of any degree or diploma. Information derived from the published or unpublished work of others has been duly acknowledged in the text.

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CERTIFICATE

This is to certify that the thesis titled "**Effect of topical anti-glaucoma medications on ocular surface: a prospective cohort study**" is the bonafide work of **Dr. Krati Srivastava** under my guidance and supervision, in the Department of Ophthalmology, All India Institute of Medical Sciences, Jodhpur.

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Dr. Krati Srivastava

LIST OF ABBREVIATIONS

AIIMS	All India Institute of Medical Sciences
AS-OCT	Anterior Segment- Optical Coherence Tomography
DED	Dry Eye Disease
OSD	Ocular Surface Disease
IOP	Intraocular Pressure
POAG	Primary Open Angle Glaucoma
TFOS	Tear Film and Ocular Surface Society
DEWS	Dry Eye Workshop
BAK	Benzalkonium chloride
OCT	Optical Coherence Tomography
OSDI Score	Ocular Surface Disease Index Score
FTBUT	Fluorescein Tear film Break-up Time
LLT	Lipid Layer Thickness
SBNFLD	Sub Basal Nerve Fiber Layer Density
TMH	Tear Meniscus Height
TMD	Tear Meniscus Depth
TMA	Tear Meniscus Area
CCT	Central Corneal Thickness
SPSS	Statistical Package of Social Sciences

IQR	Inter Quartile Range
SD	Standard Deviation
TBUT	Tear film Break Up Time
MGD	Meibomian Gland Disease

SYNOPSIS

Glaucoma is the second leading cause of blindness globally[1][2][3] and is defined as a group of progressive optic neuropathies with characteristic excavated appearance of the optic disc, (described as *cupped)* , along with loss of retinal ganglion cells and their axons and corresponding vision loss.[4] Primary open-angle glaucoma is the most prevalent of the open-angle glaucomas[5] but POAG remains a challenge to diagnose and treat since the early disease is usually asymptomatic and as the symptoms are noticeable to the patient the disease is mostly advanced. Hence, a better understanding of risk factors allows for early detection and more targeted anti-glaucoma therapy.

The raised intra ocular pressure (IOP) has direct relation to degree of optic nerve damage.[6] Amongst the various causative factors, IOP is the main modifiable factor in etiology of primary open angle glaucoma.[6] The IOP can be lowered medically ,by laser or by surgery. Topical intraocular pressure (IOP) lowering medications are the mainstay of treatment in initial stages of glaucoma.[1] In most of the patients, the treatment usually involves lifelong daily application of one or more eye drops from one-to-many times per day. Repeated dosing of topical anti-glaucoma medications can cause adverse effects to the ocular surface, and therefore triggering OSD.

Ocular surface disease (OSD) is a multifactorial disorder of tear film, eyelids, cornea and conjunctiva, characterized by inadequate quantity of tears, an unstable tear film, and ocular surface breakdown, leading to visual interference.[7] Symptoms of OSD can be irritation, burning, itching, tearing, stinging, and fluctuating visual acuity.[7] OSD is a symptomatic condition, which may affect the patient's quality of life and compliance to anti-glaucoma therapy. Signs associated with the ocular surface disease include decreased in tear break-up time (TBUT), conjunctival hyperemia, dysfunction of the Meibomian gland, and superficial punctate keratitis.[8]

Approximately 40% of glaucoma patients require more than one IOP lowering agent daily[9], resulting in longer duration of exposure to the active ingredients and preservatives in topical anti-glaucoma medications. All the components of topical anti-glaucoma medications, including the active ingredients, the preservatives as well as the excipients, may be involved in the occurrence of ocular surface toxicity.[1] There is strong evidence suggesting that glaucoma medications may contribute to occurrence of ocular surface disease (OSD) and the

development of dry eye. Studies have shown that OSD is prevalent among glaucoma patients who require long-term therapy with intraocular pressure lowering medications.[10][11][12] The severity of OSD symptoms is positively correlated with the number of intraocular pressure (IOP) lowering medications used.[13]

OSD has an age-dependent prevalence, with approximately 11% of patients between the age group of 40 and 59 and 18% of patients being older than age 80 years of age.[14] The prevalence of primary open-angle glaucoma also increases with age, approximately 1% among patients between 40–49 years of age to approximately 8% among patients being older than 80 years of age.[11] Therefore, OSD and glaucoma are both prevalent in the elderly. According to a report, 66% of subjects with severe OSD also have glaucoma. This overlap between these 2 conditions suggests an interaction between the 2 conditions or their therapies. Ocular surface disease prevalence is estimated to be about 15% among people older than 65 years, the percentage increases to 59% in patients with glaucoma.[8]

Our study aims to differentiate that whether dry eye in glaucoma patients is because of topical anti-glaucoma medications or glaucoma itself which is further aggravated by topical AMG.

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INTRODUCTION

INTRODUCTION

Primary open-angle glaucoma is a progressive optic neuropathy that is mostly associated with raised intraocular pressure (IOP) and that may lead to progressive visual field loss resulting in blindness.[15] According to the European Glaucoma Society Guidelines, the first-line approach to control IOP is with medical treatment.[15]A series of large, multicenter, randomized controlled studies showed that IOP-lowering therapy could prevent glaucoma onset from ocular hypertension and prevent further progression in patients with confirmed diagnosis of glaucoma.[16][17][18] Due to the progressive nature of the disease, long-term treatment is required, with many patients using more than one drug to achieve the target IOP.[15]

Long term anti-glaucoma therapy is not free from side effects. Ocular surface disease is one of the major side effects of topical AGM. Causes of ocular surface disease due to anti-glaucoma medications are thought to be multifactorial.[19][20][21] It can be due to either an allergic or a toxic mechanism.[19][22] Allergic responses are comparatively rare and more common in the early phases of therapy.[22][23] Hence, toxicity is likely to be the common cause of chronic ocular surface disease[19][22], which can be due to preservatives in topical AGM or active compounds itself.[15] Benzalkonium chloride (BAK) is the most commonly used preservative in topical AGM[15] and it has been identified as an independent risk factor for development of OSD in glaucoma patients.[24][25] It destabilizes the tear film lipid layer due to its detergent like tensioactive effects, which promotes excessive evaporation of aqueous tear film. Also, pro-infalmmatory and toxic effects of BAK leads to chronic ocular surface damage and disruption of tear film homeostasis.[24][25][26]

Subjective severity of Dye eye disease (DED) can be assessed by various questionnaires available while damage to ocular surface can be evaluated by a series of objective tests which include Schirmer test, TBUT, ocular surface staining, tear meniscus assessment, tear osmolarity assessment and conjunctival impression cytology.

Our study tried to evaluate whether dry eye in glaucoma is due to topical anti-glaucoma medications and their preservatives or glaucoma itself being aggravated further by topical AGM. By doing so we can detect dry eye in glaucoma patients at an early stage which will result in better and effective management of dry eye as well as increase compliance, adherence to glaucoma therapy. This in turn will lead to better long-term management of

glaucoma patients with overall improvement in the quality of life of glaucoma patients. In our study in addition to routinely performed dry eye tests like Schirmer'test, TBUT, ocular surface staining we also included tear meniscus assessment by AS-OCT and conjunctival impression cytology which was done in very few studies.

AIMS AND OBJECTIVES

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RESEARCH QUESTION: Is there any difference between ocular surface changes, anterior segment optical coherence tomography (OCT) and conjunctival impression cytology findings, corneal and conjunctival staining in the newly diagnosed cases of adult primary open angle glaucoma and adult patients of primary open angle glaucoma on topical anti-glaucoma medications.

NULL HYPOTHESIS: There is no difference between ocular surface changes, anterior segment optical coherence tomography (OCT) and conjunctival impression cytology findings, corneal and conjunctival staining in the newly diagnosed cases of adult primary open angle glaucoma and adult patients of primary open angle glaucoma on topical anti-glaucoma medications.

RESEARCH HYPOTHESIS: There is difference between ocular surface changes, anterior segment optical coherence tomography (OCT) and conjunctival impression cytology findings, corneal and conjunctival staining in the newly diagnosed cases of adult primary open angle glaucoma and adult patients of primary open angle glaucoma on topical anti-glaucoma medications.

OBJECTIVES:

1.To compare the values of Schirmer test type 1, tear film break up time test, corneal sensitivity and Ocular Surface Disease Index (OSDI) questionnaire among the newly diagnosed cases of adult primary open angle glaucoma and adult patients of primary open angle glaucoma on topical anti-glaucoma medications.

2.To compare the AS-optical coherence tomography findings among the newly diagnosed cases of adult patient of primary open angle glaucoma on topical anti -glaucoma medications.

3.To compare conjunctival impression cytology findings among the newly diagnosed cases of adult primary open angle glaucoma and adult patients of primary open angle glaucoma on topical anti-glaucoma medications.

4.To compare corneal and conjunctival staining findings among the newly diagnosed cases of adult primary open angle glaucoma and adult patients of primary open angle glaucoma on topical anti-glaucoma medications.

REVIEW OF LITERATURE

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According to Tear film and ocular society (TFOS) DEWS II dry eye disease is a multifactorial disorder of the ocular surface which is characterized by tear film homeostasis loss, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles.[27] Major differences from the TFOS DEWS definition in 2007 are recognising loss of tear film homeostasis as a key factor of dry eye diseases along with acknowledgment of neurosensory abnormalities as an causative factor.[27] To classify the DED, it is essential to identifying the primary DED subtype, i.e., aqueous deficient or evaporative but TFOS DEWS II says that the subtypes of DED rather than being distinct pathophysiological entities are part of a spectrum of disease. More importantly identifying the underlying aetiology can be useful in deciding the primary management.[27] DED prevalence ranges from 5 to 50%. Signs of DED has a higher prevalence than that of symptoms.[28]

The tear film has a precorneal thickness of approximately 2.0 to 5.5 μm and it overlies the conjunctiva and cornea.[27] Traditionally, tear film has been divided into three layers.

1. Lipid layer is the most superficial layer of tear film and is about 0.11 μm thick. It is produced by the meibomian glands. Major functions of this layer is to retard evaporation, aids in smooth movements of the lids, prevents contamination of the tear film.[29]

2. Aqueous layer is the middle layer of tear film and is the widest of all the layers. It constitutes over 90% of tear film. Thickness is approximately 7.0 μ and is the produced by the main lacrimal gland as well as accessory lacrimal glands . Most common cause of dry eye is the aqueous tear deficiency.[29]

3. The mucin layer which is about 0.02-0.05 μ thick is closest to the cornea and is produced by conjunctival goblet cells. Mucin layer helps to lower surface tension of tear film and it forms loose adsorptive coating thus rendering the corneal and conjunctival surfaces wettable.[29]

According to TFOS DEWS II , the tear film as a 2- layered interactive structure which is composed of a mucoaqueous layer and a lipid layer.[27] The mucoaqueous layer reduces the

friction and hydrates the ocular surface. The lipid layer reduces the surface tension of tear film, minimizes the evaporation and tear film instability.

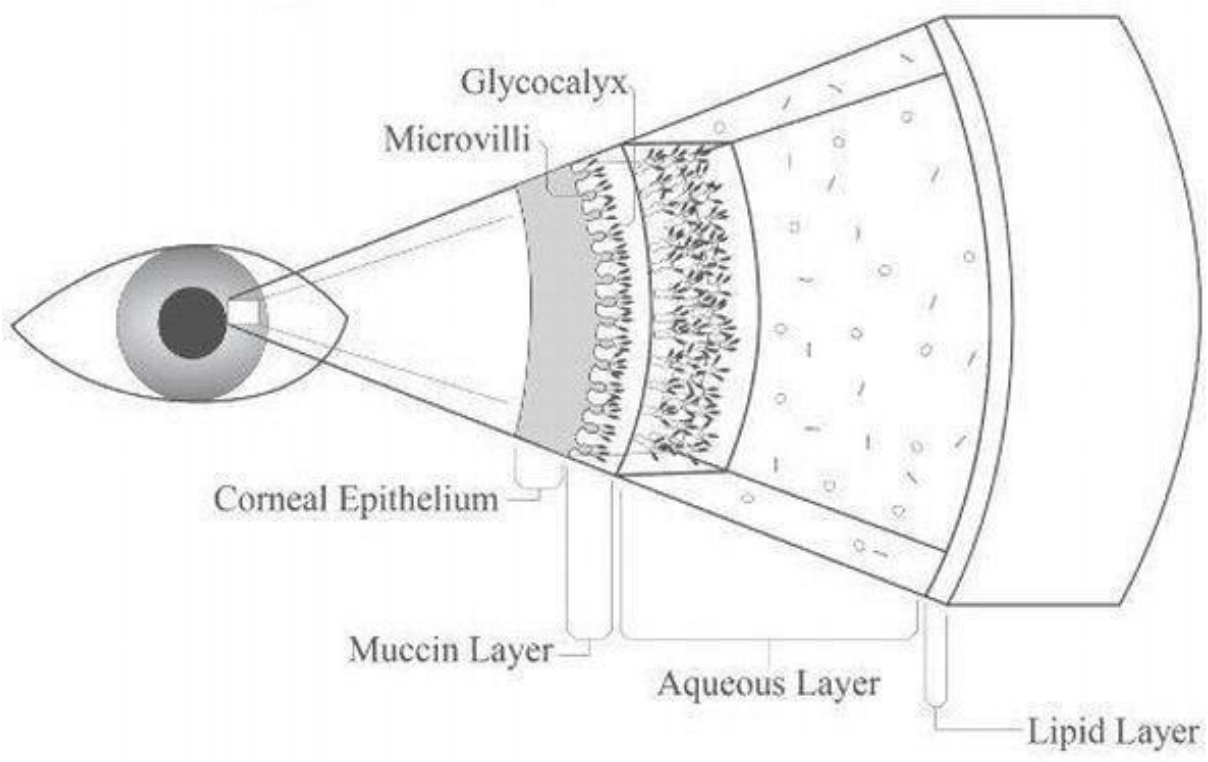

Figure 1: Layers of Tear Film
(Adapted from https://images.app.goo.gl/d9jZ6YDtVRcMRa8F8)

Dry eye disease is classified into two major catogories :

1) Aqueous deficient dry eye (Tear deficient dry eye) where the lacrimal component of tear is deficient. It can occur in the setting of advanced age, sensory reflex block, use of systemic drugs , inflammatory infiltration in conditions like Sjögren syndrome or because of lacrimal duct obstruction as seen in cicatricial conjunctival disease.

2) Evaporative dry eye (EDE), where the excessive evaporation is the main factors. It can occur in the setting of meibomian gland dysfunction, since meibomian glands are the main source of lipid layer of tear film. EDE can also occur due to certain environmental conditions, use of contact lens, decreased blink frequency.

Development of DED is a vicious circle. The loss of homeostasis of the ocular surface is the major pathophysiologic change in DED. Evaporative losses results in frictional damage to the lids and ocular surface, exacerbating hyperosmolarity.[27] Hyperosmolarity at the ocular surface in turn initiates the inflammatory cascade resulting in damage to epithelial cells, goblet cells, and tear film instability and eventually breakup. All types of DED enters the final common vicious circle of inflammation which leads to clinical signs and symptoms of DED.

Symptoms includes dryness, grittiness, scratchiness, irritation, burning, watering, soreness, and eye fatigue. In addition to symptoms, the clinical protocol for DED diagnosis requires the presence of at least one abnormal homeostatic marker. Signs are positive if both or either of the eyes has a tear film breakup time (TBUT) of ≤10s, a Schirmer test score of ≤10 mm, a Rose Bengal staining score of ≥1, a Lissamine green staining score of ≥1 .[29] Meibomian gland features and tear meniscus assessment can help to classify the predominant DED subtype and severity and to direct initial management.

At present we do not have any single gold standard test for establishing a clinical diagnosis of dry eye disease and it is well known that there is a poor correlation between the signs and symptoms of DED.

Below mentioned are various subjective and objective clinical tests that can be used to make the diagnosis of dry eye disease:

1. A number of validated questionnaires have been developed to assess the subjective experience of dry eye symptoms in patient which can help to established a more objective and reproducible data that can aid in better management and treatment of patients with dry eye disease. Currently the most commonly used questionnaires are the National Eye Institute Visual Function Questionnaire-25 and the Ocular Surface Disease Index because of they are mutlidemsional and assess the quality of life changes in the patient of dry eye disease.

A) National Eye Institute created the NEI-VFQ25 to determine the impact of visual impairment on DED patient's current health-related quality of life. This questionnaire includes the questions that deals with irritation in and around the eye. It is a 25-item questionnaire which scores on a scale of 0 to 100 points. A score of 0 is the worst score, and a score of 100 is the best score. They derived this questionnaire from a 51-item version of the

NEI-VFQ. The 25-item questionnaire has an advantage over original questionnaire since it takes shorter time to fill this as compared to the original one while retaining the multidimensionality, reliability, and validity of the original one making it better for use in clinical settings and research.

B) OSDI questionnaire is a gold standard questionnaire which was developed by the Outcomes Research Group at Allergan Inc. This is a 12-item questionnaire which evaluates the dry eye symptoms and the effects they have on vision-related function of patient's life in the past one week. Ocular symptoms, vision-related function, and environmental triggers are the 3 subscales of this questionnaire. Patients rate their responses on a 0 to 4 scale with 0 representing to "none of the time" and to "all of the time." A final score ranges from 0 to 100 with scores of 0 to 12 denoting normal, 13 to 22 denoting mild dry eye disease, 23 to 32 denoting moderate dry eye disease, and greater than 33 denoting severe dry eye disease in a patient. According to few studies a statistically significant inverse correlation exists between the tear film break up time and OSDI score. This questionnaire has a good specificity (0.83) and a moderate sensitivity (0.60) when differentiating between the patients with dry eye disease and normal subjects.

C) McMonnies Questionnaire was developed by McMonnies et al. to screen for symptoms of dry eye and to assess their risk factors. It has 14 questions that mainly focuses on the risk factors of dry eye. Gender, age, dry eye symptoms ,history of contact lens use, secondary symptoms, medical conditions associated with dry eye syndrome, dryness of mucous membranes, medication history, and any history of previous dry eye treatments are the risk factors included in this questionnaire. This questionnaire also helps to detect the patients at risk for developing dry eye disease due to exposure to certain factors that the instrument indicates.

D) Standard Patient Evaluation of Eye Dryness Questionnaire (SPEED) was designed by Korb and Blackie. Score in this questionnaire ranges from 0 to 28. The questionnaire also evaluates whether these symptoms were not problematic, tolerable, uncomfortable, bothersome, or intolerable. The questionnaire also assessed the changes in symptoms diurnally and over the period of 3 months. This questionnaire has a sensitivity of 0.90 and a specificity of 0.80.

2. Corneal sensation can be measured in all the quadrants of eye by using a cotton tip applicator (qualitative method) or more precisely with a Cochet-Bonnet esthesiometer (quantitative method).

3. Tear break up time (TBUT) is an indicator of tear film stability. After instilling a drop of topical anesthetic agent TBUT test is performed by application of fluorescein dye in inferior palpebral conjunctiva after the gentle depression of the lower eyelid. Then the patient is asked to blink several times for uniform spread of dye. Tear film is then examined with slit lamp under cobalt blue filter. The time interval between the last blink and the appearance of a first hypo-fluorescent spot or streak is recorded as the TBUT. TBUT less than 10 s is abnormal and graded as: >10 s= normal (grade 0);6.1-10 s= fair (grade 1); 3.1-6 s= moderate (grade 2); <3 s= poor (grade 3).

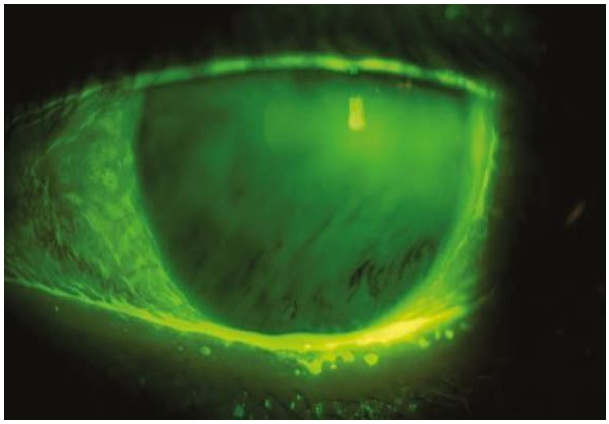

Figure 2: TBUT under cobalt blue filter
(Adapted fromhttps://images.app.goo.gl/My45n9pz13hoHSue7)

4. Ocular surface staining

Following dyes are available clinically for ocular surface staining:

A) Sodium fluorescein dye ($C_{20}H_{10}Na_2O_5$) is an orange coloured fluorescent dye with a peak excitation range between 450-500nm and it fluoresces at 500-600nm.[30] Main clinical use is to visualize the tear film stability and to highlight the ocular surface epithelium integrity. It is seen with slit lamp biomicroscope under cobalt blue filter.

Figure 3: Sodium fluorescein strips
(Adapted from https://images.app.goo.gl/71FjkDsJfnfGgiYZ7)

B) Lissamine green dye ($C_{27}H_{25}N_2NaO_7S_2$) is a greenish-blue coloured non- fluorescent dye with a molecular weight of 577g/mol. Slit lamp biomicroscope is used to visualize this staining either with the help of white light which produces a blue-green stain, or with the use of red barrier filter that results in a black appearance to the pattern of ocular staining.

Figure 4:Lissamine green strips
(Adapted from https://images.app.goo.gl/PiajGFHeca32cvJw5)

C) Rose bengal ($C_{20}H_2Cl_4I_4Na_2O_5$)dye was introduced in 1903 by Schirmer. It is a disodium salt with a molecular weight of 1018g/mol and it binds to cell nuclei. It is also seen with slit lamp biomicroscope under white light with no barrier filters.

Figure 5: Rose Bengal strip
(Adapted from https://images.app.goo.gl/6JiUQLi3Ncbo8ty5A)

A number of grading scales area present to evaluate the ocular surface staining , although no "gold standard" universally accepted grading scale exists for corneal and conjunctival staining.[31]

The most commonly used scales in clinical practise are:

A) NEI (National Eye Institute) grading scale consists of a grid which divides the cornea into five parts and conjunctiva into six parts. Each part is assigned a score between zero and 3 depending on the amount and distribution of staining. The total score ranges from 0 (denoting the absence of corneal epitheliopathy) to 15 (denoting severe epitheliopathy).

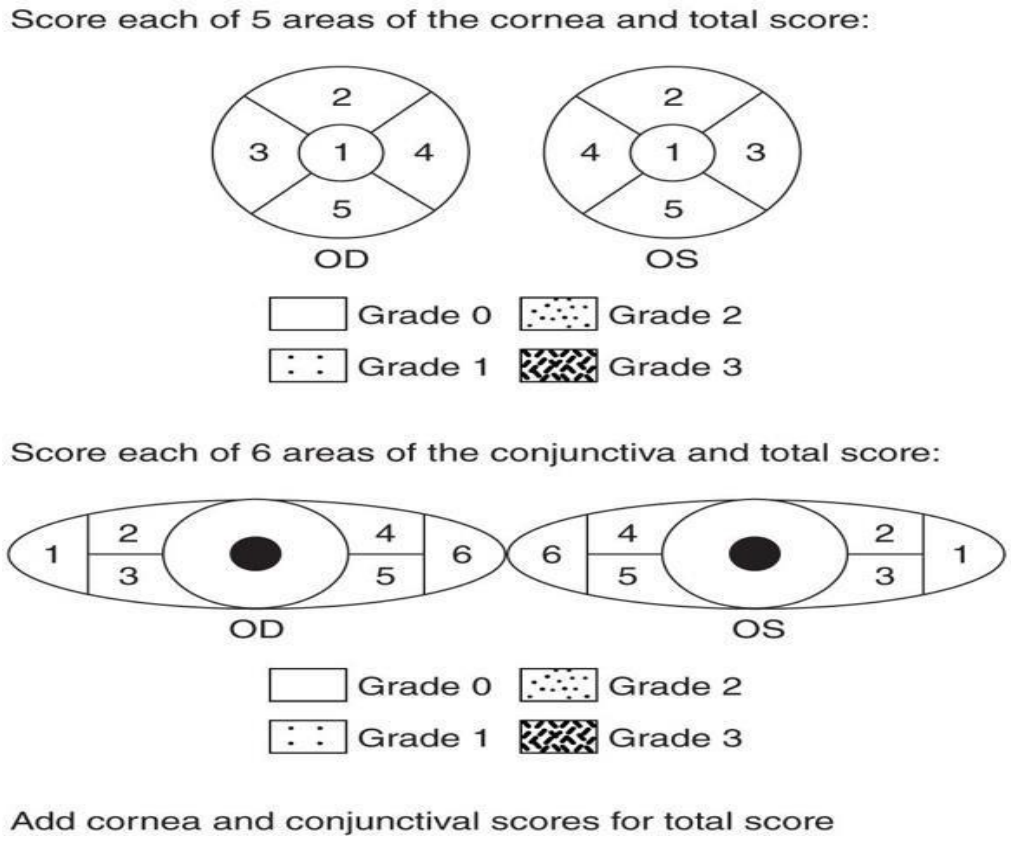

Figure 6: NEI ocular grading

(Adapted from https://images.app.goo.gl/LiGc4Qc4DLYyABXCA)

B) Oxford grading scale consists of a series of panels which are labeled from A–E, in increasing order of severity. This scale reproduces the staining patterns that are commonly encountered in dry eye disease patient and are used as a guide to grade the amount of staining observed in the patient.[32]In each panel, the amount of staining is represented by punctate dots. The number of dots seen increases by 1 log unit between panel A and B and by 0.5 log units between rest of the panels from.[32] Staining is graded by comparing the panels and the appearance of staining seen on the cornea and exposed interpalpebral conjunctiva of the patient. No attempt should be made to count the dots seen or to assess the confluence or position of the dots.

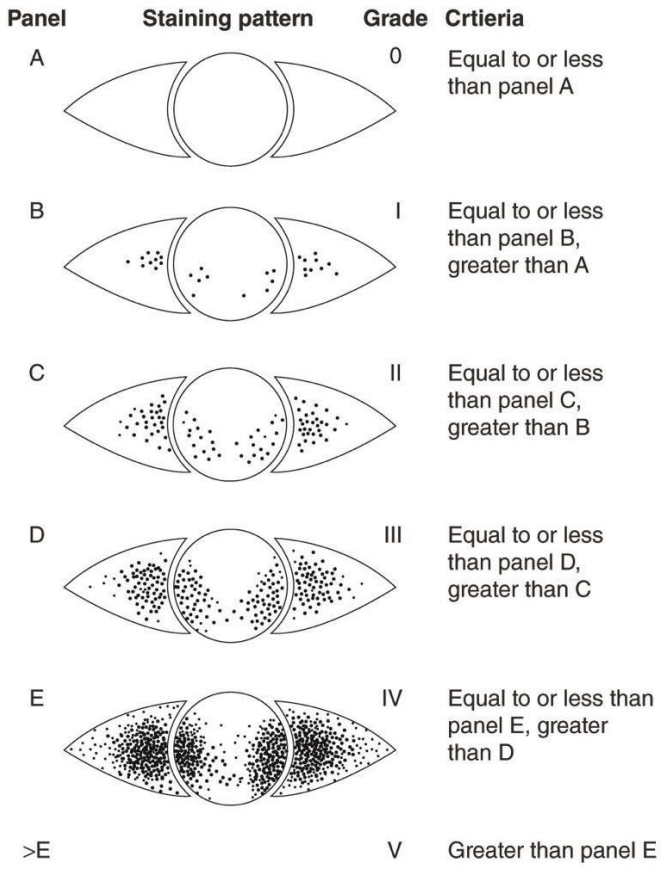

Figure 7: Oxford grading scale

(Adapted from https://images.app.goo.gl/79pxf3gP7Z6aS8TDA)

3. In the van Bijsterveld schema, the in-tensity of lissamine green (a dye that stains devitalized epithelial cells and has replaced Rose Bengal) is scored in the two exposed conjunctival zones and cornea are scored between 0□3, giving a maximum score of 9. In the van Bijsterveld schema, the in- tensity of lissamine green (a dye that stains devitalized epithelial cells and has replaced Rose Bengal) is scored in the two exposed conjunctival zones and cornea are scored between 0-3, giving a maximum score of 9

C) Van bijsterveld score was published by Van Bjisterveld for an ocular surface grading in keratoconjunctivitis sicca almost 50 years back. Rose bengal dye was used originally to stain the ocular surface. A modification to the score was introduced with the 2002 revised American European Consensus Group (AECG) criteria which allowed the use of fluorescein

dye to stain the cornea and the use of lissamine green dye to stain the conjunctiva because rose bengal is associated with significant patient discomfort.[33] In this staining score the entire cornea and the nasal and temporal conjunctiva are scored using a 0-3 grading system based on increasing intensity of staining. According to AECG criteria, a Van bijsterveld score of 4 or more in either eye is considered positive.[34]

D) The OSS was calculated for each eye as follows: for the cornea, the fluorescein staining is used with score ranging from 0-3 score, 0 if punctate epithelial erosions are absent , 1 if 1-5 erosions are seen ; 2 if 6-30 erosions are noted and for ≥ 30 erosions, the score of 3 is given. An additional point is added to the corneal score for each of the following (if present): erosions seen in the central 4 mm of the cornea (pupilar staining), the presence of ≥1corneal filaments, or areas of confluent staining. Lissamine green staining is used to stain temporal and nasal conjunctiva and is scored similarly, with a grade of 0-3.New ACR/EULAR criteria uses a threshold of 5 or more for a positive OSS in Sjogren syndrome.[34]

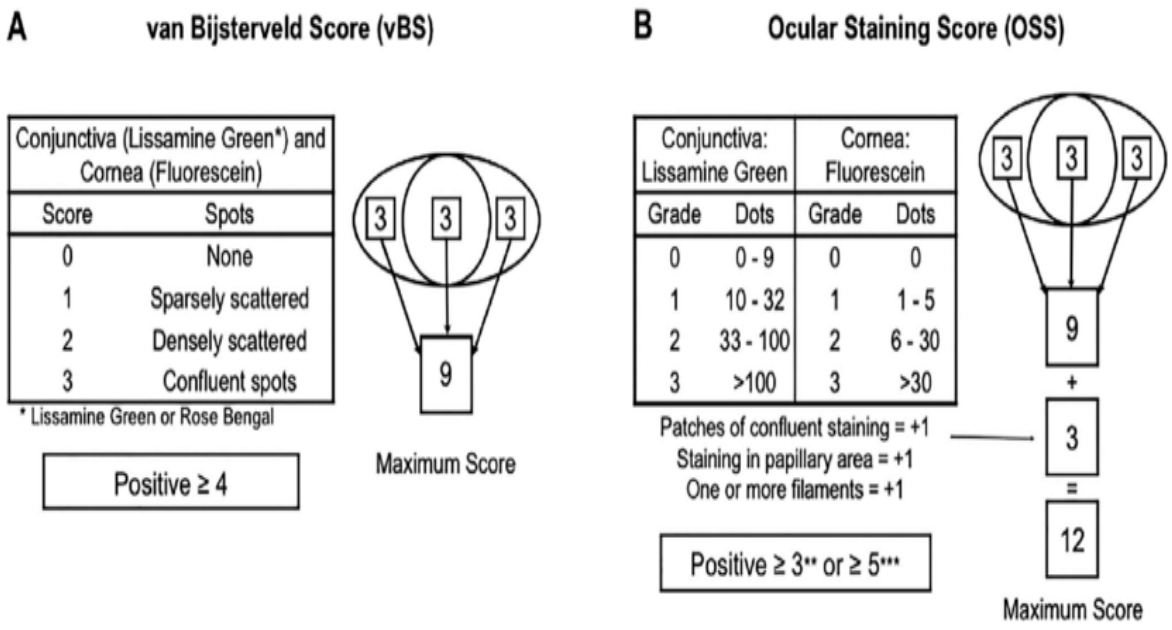

Figure 8: Van bijsterveld score and Ocular surface score
(Adapted from https://images.app.goo.gl/T4rwxyiUv7eUf92s6)

In the present scenario, the Oxford and NEI scales are the most commonly used in clinical trials to grade the ocular surface staining because of the systematic methodology they use.

Despite being more systematic, these ocular surface staining scales are still observer dependent and subjective, and therefore are susceptible to high interobserver and intraobserver variance.

5. Schirmer test is done using Whatman filter paper no. 41 which measures 40×5 mm with markings from 0 to 35 mm. It can be done with and without topical anaesthesia. Schirmer test type I is done without anaesthesia and it measures both basal and reflex lacrimation while Schirmer test type II is done with anaesthesia but is done following nasal stimulation. It measures only reflex lacrimation and it has been seen that it is reduced more in Sjögren's syndrome compared to non-Sjögren's dry eye. The Schirmer test is done by placing a schirmer's strip at the junction of medial 2/3rd and lateral 1/3rd of lower eyelid after gently pulling the lower lid down. Then the length of the moistened portion of the strip after 5 minutes is measured and graded as: >10 mm= normal (grade 0); 5-10 mm= mild (grade 1); 3-4 mm= moderate (grade 2); 0-2 mm= severe (grade 3).

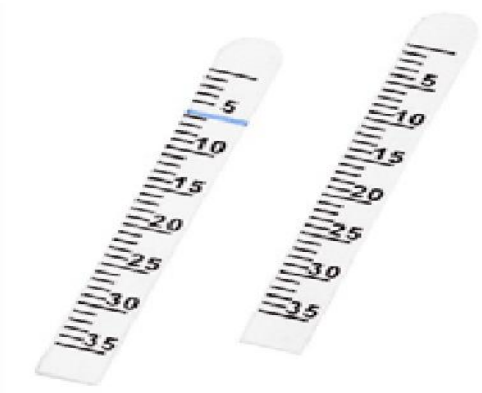

Figure 9: Schirmer Strips
(Adapted from https://images.app.goo.gl/pBfTnCfxgygZZP8Q6)

6. It has been proposed by many studies that topical AGM exerts adverse effects on the conjunctiva.[35][36] As most of the glaucoma patients will eventually require the filtration surgeries.[37] Presence of a healthy conjunctiva is one of the pre-requisites for the successful outcome of filtration surgeries. Conjunctival inflammation and scarring due to long term use of topical AGM, predisposes the patient to poor outcome after glaucoma filtration surgery.[38][37] Conjunctival impression cytology is a safe, painless tool to obtain cytologic specimens of conjunctiva to assess the changes in conjunctiva. It can be used both as a

diagnostic and prognostic test in assessment of ocular surface. Impression cytology specimens are obtained with the help of nitrocellulose membrane filter (pore size 0.22μ).After fixing the smear with 95% ethyl alcohol, the smears are stained with Papanicolau stain and/or Periodic Acid Schiff stain and graded accordingly.

7. Assessment of tear meniscus

A vital role is played by tear meniscus in tear dynamics because it has been reported that tear meniscus holds around 75–90% of the total tear film volume.[39] Modifications that tear meniscus (TM) undergoes in the presence of ocular surface diseases (OSDs) are extremely important, with TM curvature, height, width, and cross-sectional area, considered as structural indicators of EDE.[40] Tear meniscus parameters can be determined by various methods like using a reticule at the slitlamp, image capture, interferometry, reflective meniscometry and OCT.[41][42][43][44] Amongst all , the OCT has high sensitivity (67.0-80.5%) and specificity (81-89.3%) along with shown good repeatability (ICC = 0.900–0.981) for the diagnosis of dry eye disease.[45][46][47]

Optical coherence tomography (OCT) was first introduced in 1991 for imaging of the posterior segment of the eye[48] and 3 years after that , first anterior segment-OCT (AS-OCT) was introduced.[49] Anterior segment-optical coherence tomography (AS- OCT) is a noninvasive, noncontact, and rapid method which is able to morphologically evaluate and capture the high-resolution cross-sectional images of all the structures in anterior segment of the eye along with the ocular surface epithelium and the tear film. AS-OCT has also been utilized to analyze the precorneal tear film and the TM.[50]

OCT measures the delay of light reflected from various ocular tissues.[51] It utilizes a Michelson interferometer, which creates a reference beam , which is usually of infrared light against which multiple other beams of light are measured as they return from the differently reflective tissues of the eye. Device creates multiple interference patterns over the surface of the structure that is being imaged resulting in creation of a series of A-scans. All these A-scans are combined to create a composite cross-sectional B-scan. There are mainly two major types of OCTs available. Time domain OCT and Fourier-domain OCT. The Fourier domain

system has a advantage over time domain OCT because it provides a faster acquisition times more detailed visualization of ocular tisses due to its higher resolution.[52]

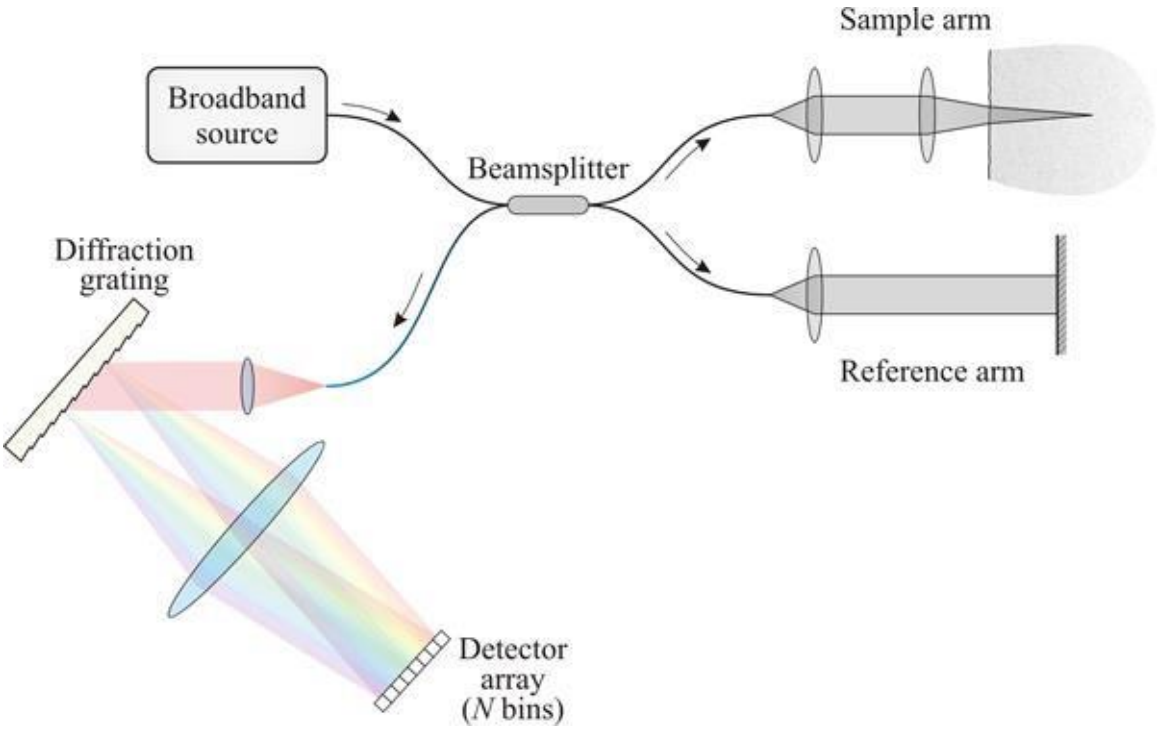

Figure 10: The Basic Principle of Optical Coherence Tomography
(Adapted from http://obel.ee.uwa.edu.au/research/fundamentals/introduction-oct/)

AS-OCT allows to capture and measure a high quality image of the tear meniscus. Tear meniscus is basically a triangular shape of wedge of tear film present between the lower lid margin and ocular surface.[52] Three parameters can be measured in the image; tear meniscus height, tear meniscus depth, and tear meniscus area. The averages of TMH, TMD, and TMA were 295.58 ± 58.36 μm, 166.67 ± 30.43 μm, and 0.0360 ± 0.01100 mm2 in normal eyes.[53] Tear meniscus height (TMH) is defined as the vertical linear length present between upper end of tear meniscus which is reaching the cornea and lower end of the tear meniscus which is reaching the lower conjunctiva.[53] Tear meniscus depth (TMD) is the horizontal linear length between the outer limit of the tear meniscus and apex.[53]

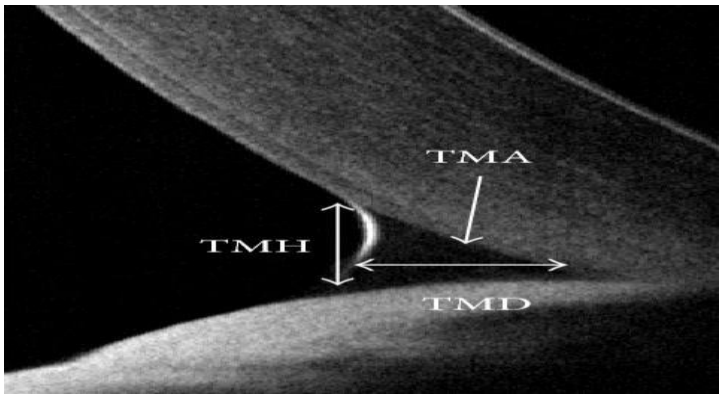

Figure 11:Tear meniscus on AS-OCT
(Adapted from https://images.app.goo.gl/hQpcUnb23HBPvCUw5)

Topical anti-glaucoma medications has a critical role in the management of glaucoma worldwide. Due to the chronic nature of the disease, most of the patients requires lifelong therapy with topical anti-glaucoma medications which leads to ocular surface disease, a frequently reported side effect of long duration topical antiglaucoma therapy. All ocular surface structures are modified during the medical management of glaucoma, including the conjunctiva, the conjunctiva-associated lymphoid tissue, cornea and corneoscleral limbus, meibomian glands (MGs), and tear film. Of note, alterations affecting these structures are similar, at least in part, to those observed in evaporative dry eye (EDE).

To reduce the risk of bacterial contamination in multi-dose bottles of anti-glaucoma eye drops, preservatives are added to them. Most commonly used preservative is benzalkonium chloride (BAK). Ocular surface disease due to topical anti-glaucoma medications can be due to either preservative used in topical AMG or active ingredient itself or both. BAK damages the DNA which results in disruption of cellular architecture of corneal epithelium, disrupts the tight junctions and induces cell death. All these events decreases the cellular viability, induces inflammatory response, and impairs the cellular proliferation. It also causes subconjunctival fibrosis and loss of goblet cells which results in worsening of quality of tear film and aggravates the symptoms of ocular surface diseases. By all the above mentioned factors, BAK negatively affects the long-term success rate of glaucoma filtering surgery.[9] It also has an adverse effect on the quality of life in glaucoma patients. Hence, the development of less toxic preservatives, preservative free topical medications, and non-drop therapy like drug-eluting systems for the delivery of ocular medications, are advocated in the better care

and quality of life in glaucoma patients along with a increased compliance which eventually leads to effective management of glaucoma and good results of glaucoma filtering surgery.

1. Pérez-Bartolomé F et al[54] conducted a study in 2017 to see the relationship between ocular surface disease (OSD) and topical antiglaucoma therapy. In the study a total of 211 eyes of 211 patients with open-angle glaucoma or ocular hypertension on topical medication were recruited over the period of 10 months. 51 eyes of 51 healthy age- and sex-matched volunteers were recruited as controls. Type of anti-glaucoma medications used , the number of medications used, and daily and cumulative preservative concentrations (PC) were recorded in all the patients. Outcome was assessed based on fluorescein corneal staining score (Oxford scale), lower tear meniscus height (LTMH) (spectral-domain optical coherence tomography), non-invasive tear film breakup time (NI-TBUT) (Oculus Keratograph 5M), and OSD symptom questionnaire index (OSDI). As compared to controls, a significantly higher OSDI (median 10.24 [4.54-18.94] vs 2.5 [0-12.5]; $p<0.001$) and corneal staining (≥1: 64.93% vs 32.61%; $p<0.001$) scores were recorded in the anti-glaucoma medication group. Although no significant difference was noted in NI-TBUT and LTMH between the groups ($p>0.05$). Lower LTMH (R -0.142; $p = 0.043$) was found in higher daily PC. Multivariate analysis in the medication group, identified correlations between benzalkonium chloride (BAK) (odds ratio [OR] 1.56) and BAK plus polyquaternium-containing drops (OR 5.09) or higher OSDI (OR 1.06) and abnormal corneal staining test results and between older age (mean ratio [MR] 1.05), longer treatment duration (MR 1.02), or corneal staining presence (MR 1.22) and a higher OSDI score. This study concluded that medication group had a higher prevalence of ocular surface disease. Drops with preservatives, longer treatment duration, and older age were the major factors impacting OSD.

2. Norlina Ramli et al[7] carried out a cross-sectional, case-comparison study between June 2012 and January 2013 to calculate the prevalence of ocular surface disease (OSD) in glaucoma and nonglaucoma subjects and to determine the effect of number of antiglaucoma medications and preservatives on OSD via various clinical tests.105 Glaucoma patients (n = 105) on topical antiglaucoma medications were compared with 102 controls who were not on any topical medications. Ocular Surface Disease Index (OSDI) questionnaire grading ,tear film breakup time (TBUT) test, corneal staining, Schirmer test were used to assess the presence of OSD. According to this study prevalence of OSD varied from 37 to 91% in the glaucoma group. In the glaucoma group subjects had corneal staining (63% vs. 36%, p =

0.004), abnormal Schirmer tests (39% vs. 25%, p = 0.049), and moderate OSDI symptoms (17% vs. 7%, p = 0.028). Use of higher numbers of topical medications was associated with increased percentage of abnormal TBUT . Increased percentage of abnormal TBUT was also seen with both benzalkonium chloride containing and preservative-free eye drops (90% and 94%, respectively, both p G 0.001). Nearly three times higher odds ratio of abnormal OSDI was associated with Benzalkonium chloride.

3. Houda Lajmi et al[55] conducted a cross-sectional study to assess the clinical and the functional findings in patients with glaucoma on preserved anti glaucoma eyedrops having ocular surface alterations and to analyze their risk factors. 155 patients with glaucoma were enrolled in the study. Each patient answered the "Ocular Surface Disease Index" (OSDI) questionnaire and underwent thorough evaluation of the ocular surface state via Schirmer I test, tear break-up time evaluation, eyelid, conjunctival, and corneal examination with a Fluorescein and a Lissamin green test. Factors (age, sex, glaucoma treatment duration, number and type of the active principle, and Benzalkonium Chloride [BAK] use) that could influence the OSDI score and each type of ocular surface alteration were assessed. BAK was present in 80% of anti-glaucoma eyedrops . In 61.3% of cases ,OSDI score was ≥13. Biomicroscopic signs of ocular surface disease were at least minimal in 87.1% of cases. Duration of glaucoma treatment (P = 0.01, t = 2.618), the number of molecules used (P = 0.018, t = 2.391), and the use of BAK (P = 0.011, t = 2.58) were the main predictors of increased OSDI score. The biomicroscopic signs severity correlated with these same risk factors . Fixed combination of anti-glaucoma eyedrop was statistically associated with a reduced incidence of superficial punctate keratitis (SPK) and corneal and conjunctival staining in the Lissamine green test (P < 0.001). Significantly higher risk of SPK and corneal or conjunctival staining in the Lissamine green test (P < 0.001) was associated with Beta-blockers class of anti-glaucoma eyedrops. Conclusion drawn from this study was that the preserved anti-glaucomatous eye drops alter the patients' ocular surface and the major risk factors were advanced age, duration of glaucoma treatment, multiple therapies, and the use of BAK.

4. Fechtner RD et al[11] carried out a prospective observational study in 2009 to look for prevalence of ocular surface disease (OSD) in patients with glaucoma using topical anti-glaucoma medications. They enrolled 630 patients with primary openangle glaucoma or ocular hypertension who were on a topical anti-glaucoma medications. For each enrolled

patient OSDI scores (0–100, with 0 representing no symptoms) were calculated .Detailed medical history, demographics, and concomitant medication use information were also obtained. Out of 630 participating patients , 305 patients (48.4%) had an OSDI score depicting either mild (n = 134, 21.3%), moderate (n = 84, 13.3%), or severe (n = 87, 13.8%) OSD symptoms. A significant difference in OSDI scores between patients with and without a prior diagnosis of dry eye syndrome (25.2 6 15.4 vs 15.4 6 15.8, respectively; P = 0.0036) and also between patients who did and did not use artificial tears at the time of participation in the study (23.0 6 15.6 vs 15.3 6 15.8, respectively; P = 0.0046) was noted. Number of topical IOP-lowering medications used was associated with significant variation in mean OSDI scores. Higher (more severe) OSDI scores were seen in patients using multiple IOP-lowering medications. Patients on one anti-glaucoma medication had a mean OSDI score of 12.9 6 13.1, which was significantly lower when compared to patients on 2 (16.7 6 17.0; P = 0.007) or 3 anti-glaucoma medications (19.4 6 18.1; P = 0.0001). Conclusion drawn from this study was that OSD is prevalent among patients with glaucoma who are treated with anti-glaucoma medications and number of IOP-lowering medications used had a positive correlation with severity of OSD symptoms.

5. A multicenter, randomized ,prospective, parallel group, single-blind clinical study was done by Russ HHet al[56] in the year 2013 to compare ocular surface changes because of glaucoma treatment in patients using fixed combinations of prostaglandin analogues (travoprost, latanoprost and bimatoprost) with 0.5% timolol maleate. The study enrolled 33 treatment naïve cases with ocular hypertension or open angle glaucoma. Ocular surface assessment was done before initiating the treatment and three months after treatment. Effect of these anti-glaucoma medications on ocular surface were evaluated via Schirmer's test, tear film break-up time, Lissamine green staining, Ocular Surface Disease Index questionnaire, immunocytochemistry for interleukin-6 and HLA-DR, impression cytology using HE and PAS. The study showed a decreasein Schirmer's test results with all of the above anti glaucoma drugs and also a decreases in tear-film break-up time test results with travoprost/ timolol and latanoprost/timolol along with increase in the Lissamine green score with travoprost/timolol and bimatoprost/timolol. In the travoprost/timolol group , Ocular Surface Disease Index score increased after treatment. In impression cytology there was a significant difference in cell-to-cell contact in the same group with an increase in cellularity and an increase in the number of goblet cells in all of the groups. An increase in IL-6 expression along with an increase in HLA-DR expression in the travoprost/timolol group

was also seen. Conclusion derived from this study was that travoprost/timolol group showed increased expression of the inflammatory markers HLA-DR and interleukin-6 and all the above fixed combinations of anti-glaucoma medications resulted in hazardous effects on the ocular surface after three months of glaucoma treatment.

6. In a study conducted by Aydin Kurna S et al[(2)] a comparative analysis of effects of various antiglaucoma eye drops as monotherapy on tear functions and ocular surface for the period of 12 months follow up was done. It was a randomized prospective study.85 eyes of 43 patients with primary open angle glaucoma who had no past history of topical antiglaucoma drugs use were enrolled in the study. Patients were divided into six groups and started on 0.5% timolol maleate(preservative free)-group 1; 0.5% timolol maleate with 0.012% benzododecinium bromide -group 2; 0.005% latanoprost with 0.02% BAK- group 3; 0.03% bimatoprost with 0.005% BAK-group 4; 0.004 travoprost with 0.015% BAK-group 5; 0.1% brimonidine with purite 0.005%-group 6.Outcome variables were conjunctival impression cytology (performed at 3rd, 6th, and 12th months for each eye) , tear film breakup time (TBUT), staining scores (SS), and Schirmer (SCH) I tests(done before starting the treatment and then at 1st week, at 1st, 3rd, 6th, and 12th months). According to this study there was no statistically significant difference in mean SCH I test results among the groups during the course of the study ($P > 0.05$)TBUT changes were absent in groups 1, 2, 3, and 6 before and after the treatment although there was a significant decrease in TBUT in group 4 at 1st week and 1st month controls ($P = 0.048$, $P = 0.0019$) and in group 5 at 1st week and 1st, 3rd, 6th, and 12th month controls ($P = 0.018$, $P = 0.004$; $P < 0.05$). Groups 1 and 2 showed higher corneal staining scores , while a higher conjunctival staining scores were seen in group 6. In groups 1 and 5 decreased goblet cell count were seen in superior and inferior quadrants while in group 2 in Goblet cell count decreased in superior quadrant and in groups 3 and 6 decrease in goblet cell count were seen in in inferior conjunctiva. A significant increase in grades of squamous metaplasia were observed in in groups 1 and 2 at 3rd, 6th, and 12th month controls ($P < 0.05$). This study concluded that a nonserious impact on tear function tests and low-grade metaplasia with topical antiglaucoma monotherapy at the end of 12th month control was observed. Preserved and non-preserved beta blockers resulted in higher damage to the ocular surface when compared to prostaglandin analogues and brimonidine-purite. Overall this study suggested that besides preservative substances, the number of times an antiglaucoma medications used in a day and active substances are also responsible for the ocular surface changes seen.

7. Gozawa M et al[57], carried out a study to evaluate the effects of various factors in preservation rate of the borderlines of conjunctival layers in the patients with glaucoma treated with anti-glaucoma eye drops. 199 patients(328 eyes) with and without anti-glaucoma eye drops were enrolled in the study. Analysis of anterior segment optical coherence tomography (AS-OCT) images of superior bulbar conjunctiva was carried out in order to quantify the preservation rates of the conjunctival layer borderlines. This study showed lower preservation rates of the borderlines between both the conjunctival stroma/Tenon's capsule ($P < 0.001$ and $P < 0.001$, respectively) and Tenon's capsule/sclera ($P < 0.001$ and $P < 0.001$, respectively)were associated with increase in number of anti-glaucoma eye drops and a longer duration of anti-glaucoma medications used. Prognostic factors for lower preservation rates of the borderlines between both the conjunctival stroma/Tenon's capsule ($P < 0.001$ and $P = 0.009$, respectively) and Tenon's capsule/sclera ($P < 0.001$ and $P = 0.008$, respectively) were prostaglandin analogs and fixed combinations of β-blockers/prostaglandin analogs while a significantly higher preservation rates were seen with α2-receptor agonist ($\beta = 0.103$, $P = 0.021$) when compared to other anti-glaucoma eye drops. Conclusion derived from the study was that the several anti-glaucoma eye drops and their long-term administration are responsible for disruption of the bulbar conjunctival borderlines as detected by AS-OCT.

8. A Cross-sectional comparative study conducted by Hong S et al[58] to determine the effect of various topical antiglaucoma eye drops on conjunctival impression cytology specimens. The study enrolled a total of 170 patients (170 eyes) with primary open-angle glaucoma and 20 normal subjects (20 eyes). All patients with primary open-angle glaucoma were treated for six months (timolol n 34; latanoprost n 40; dorzolamide n 32; timolol+ latanoprost n 30; timolol +dorzolamide n 34). No ophthalmic abnormalities were present in controls and they did not receive any topical anti-glaucoma eyedrops. Impression cytology specimens were collected from inferior bulbar conjunctiva of the eyes taking no topical anti-glaucoma eyedrops (controls= n 20) and from the eyes taking antiglaucoma medications. Nelson's grading was used to grade the specimens on a scale of zero to three. A significantly higher cytology scores were obtained in the medication group as compared to the control group. Mean cytology scores of the control, timolol, latanoprost, dorzolamide, timolol latanoprost, and timolol dorzolamide group were 0.20, 1.62, 2.00, 1.75, 2.13, and 2.44, respectively. A significantly higher cytology scores were seen in fixed-combination therapy group when compared to the monotherapy group. A considerable degree of squamous

metaplasia was associated with longer duration of use of Several commercially available topical antiglaucoma eyedrops .

9. To evaluate the tear meniscus in medically controlled primary open angle glaucoma patients with the help of anterior segment-optical coherence tomography (AS-OCT) an interesting case-control, single-center, observational study was done by Agnifili L et al[50] in 2019.They included 56 medically controlled primary open angle glaucoma patients, 24 patients with evaporative dry eye (EDE), and 30 healthy subjects (controls) . Medically controlled primary open angle glaucoma was further subdivided into group 1 :β-blockers(n= 14 eyes); group 2 (: prostaglandin analogs (n= 14 eyes) ; group 3 : ≥2 drugs (n= 28 eyes) Ocular Surface Disease Index (OSDI) questionnaire, Schirmer Test I ,tear film break-up time test , corneal fluorescein staining and tear meniscus height (lower and upper: L-TMH, U-TMH) and area (L-TMA, U-TMA) via AS-OCT, were performed in all the enrolled patients.A significantly higher OSDI score was observed ($P < 0.05$) in patients with evaporative dry eye and in group 3 when compared with groups 1, 2, and controls. In groups 1 to 3 ($P < 0.05$) and in EDE patients ($P < 0.001$) L-TMA was significantly lower as compared with controls, and it was also significantly lower in group 3 and in EDE patients when compared to groups 1 and 2 ($P<0.05$). Similar results were seen in L-TMH. In groups 1 to 3 and in EDE patients , L-TMH was lower as compared to controls ($P < 0.001$), and in EDE patients and in group 3 compared with groups 1 and 2 ($P<0.05$). A lower U-TMA was observed in EDE and MCGP groups compared with controls ($P < 0.05$). There was a negatively correlation of L-TMA and L-TMH with OSDI score ($P < 0.01$, $r=-0.379$ and $P<0.01$, $r=-0.352$, respectively).Between group 3 and patients with EDE there were no significant differences in all the clinical parameters. AS-OCT is a non-invasive , quick and reliable modality for imaging of tear meniscus in medically controlled glaucoma patients demonstrating the glaucoma- related ocular surface disease as a dry eye disease–like condition. Therefore, a reduction values of TMH and TMA can be considered as a structural indicators of topical anti-glaucoma (eyedrops) therapy–related ocular surface disease.

10. Another prospective, cross-sectional study to assess the quality of life in glaucoma patients with the help of Ocular Surface Disease Index (OSDI) questionnaire and their relationship with dry eye clinical signs was done by Guarnieri A et al.[8]A total of 209 patients participated in the study. Out of the 209 patients, 147 patients diagnosed with bilateral open-angle glaucoma and were on one or more anti- glaucoma eyedrops in both eyes

for at-least 1 year, 21 patients had a history of bilateral glaucoma filtration surgery without any topical anti- glaucoma eyedrops ion either of the eyes (at least 12 months before the study). 41 subjects were taken as controls. All the enrolled participants ,filled the OSDI questionnaire along with evaluation of dry eye signs via 3parameters- (1) non-invasive tear break-up time (NITBUT),(2) conjunctival hyperaemia , (3) Meibomian gland depletion . All the above parameters were measured using the Keratograph5 M . The total-OSDI (T-OSDI) score was subdivided into discomfort-OSDI scores and visual field-OSDI scores. A higher T-OSDI and sub-scores was found in glaucoma patients (both medically treated and surgically operated glaucoma patients) when compared with controls (p \ 0.05); but there was no difference between group treated with anti-glaucoma medications and the group which underwent glaucoma filtration surgery. Positive correlations were noted amongst T-OSDI values and Schirmer test (p = 0.016), ocular surface staining (p \ 0.001) and the MGD (p = 0.006).The OSDI subscores were associated with the ocular surface staining (VF p = 0.013 and D p = 0.003). In medically treated patients, the number of drops per day positively correlated with T-OSDI and OSDI subscores (p = 0.017 and p = 0.005). Conclusion derived from this study OSDI scores are increased in the glaucoma patients (both medically treated and surgically operated glaucoma patients) as compared to controls without any significant differences between medically treated and surgically operated glaucoma patients. A higher OSDI scores were associated to more severe dry eye signs andante-glaucoma medications.

11. A prospective observational study conducted by Pai, V et al[(59)] during the period between August 2013 and September 2015 to determine the difference in prevalence of ocular surface disease (OSD) in patients on antiglaucoma eyedrops vs normal subjects .The study comprised a total of 188 participants, out of which 94 patients with glaucoma (primary open angle glaucoma, primary angle closure glaucoma, combined mechanism glaucoma, pseudoexfoliation, and pigment dispersion glaucoma) on topical antiglaucoma eyedrops for a period of more than six months constituted the study group while 94 age- and gender-matched normal subjects who were not on any topical medications constituted the control group. All the participants were evaluated for ocular surface disease through ocular surface disease index (OSDI) questionnaire, tear break-up time (TBUT), Lissamine green staining of cornea and conjunctiva, and Schirmer's test I. According to this study the group with participants on anti-glaucoma eyedrops (72.4%) had a significantly higher prevalence of OSD when compared to controls (44.6%) using OSDI questionnaire.84% patients had reduced tear production in the study group vs 53% in controls as depicted by Schirmer's test

I. An abnormal TBUT was found in 67.1% of the participants in the study group and of 47.8% control group.36.2% of patients in the study group while 31.8% of controls had a was positive Lissamine green staining of cornea and conjunctiva. This study derived a Conclusion that ocular surface disease was more prevalent in patients on intraocular pressure (IOP)-lowering eyedrops than in controls and also a longer duration of anti-glaucoma therapy and multiple IOP lowering eyedrops were associated with more severe OSD.

12. Kamath AP et al[6] did a prospective cohort study in order to see the changes in conjunctival epithelium caused by long term use of topical anti-glaucoma therapy. 100 cases of primary open angle glaucoma were included in the study and they were divided into four groups of 25 patients each. Group 1 started on eyedrop Timolol, group 2 on eyedrop Pilocarpine, group 3 on eyedrop Brimonidine and group 4 on eyedrop Latanoprost. All the cases were followed up over the period of 1 year. Ocular surface changes due to topical anti-glaucoma were evaluated using Schirmer's test, tear film break up time (BUT) and conjunctival impression cytology, done once in 3 months. At the end of 1 year decreased Schirmer's test value was seen in 40% of cases and reduced tear film BUT values in 26% of the cases. These changes were higher in patients on timolol eyedrops. There were also a significant changes in conjunctival impression cytology that included decreased density of goblet cells , squamous metaplasia and presence of inflammatory cells. Inflammatory responses were more common with brimonidine and pilocarpine treated POAG patients treated while squamous metaplasia was more commonly seen with latanoprost eyedrops. This study concluded that conjunctival impression cytology is a non-invasive modality to assess the effect of topical anti-glaucoma medications on ocular surface and the ocular surface changes were significantly higher in patients on topical anti-glaucoma medications . These changes in ocular surface had a direct relationship to duration of treatment with anti-glaucoma medications.

13. An observational, cross-sectional study conducted by Rossi GC et al[60] to look for the signs and symptoms of ocular surface disease (OSD) in patients treated with Intra Ocular Pressure (IOP)-lowering topical medications and also to assess the relationship among the signs and symptoms of OSD; along with identification of methods to diagnose and follow a case of OSD with its impact on the quality of life. 233 patients were included in the study. 184 subjects had a diagnosis of POAG (, 78.9%), 37 patients (15.8%) presented with Ocular hypertension, 6 patients had (2.6%) pseudo-exfoliative glaucoma , 5 patients (2.2%) normal

tension glaucoma and 1 patients had (0.4%) pigmentary glaucoma. To be included in the study, the subjects should have been treated with topically administered benzalkonium chloride- preserved IOP lowering medications. A complete ophthalmic evaluation was done and tear film break-up time (TF-BUT) and fluorescein corneal staining (keratitis punctatae) were assessed in the subjects enrolled in the study. They also filled Italian version of both the National Eye Institute-Visual Function Questionnaire (NEI-VFQ) 25 and the Glaucoma Symptom Scale (GSS) questionnaires. Of the total participating patients, 70 eyes(30%) had puctate keratitis, 67 eyes(28.8%) showed abnormal TF-BUT, 97 patients (42.1%) had an OSD. The abnormal values of various ocular surface parameters were gender- independent but age($P = 0.01$) and number of instillations/die ($P = 0.0007$)had a statistically significant relation to keratitis. Statistically significant relationship was present between the NEI ocular pain subscale and TF-BUT ($P = 0.017$); GSS had a significant relationship to both theTF-BUT and punctatae keratitis ($P < 0.00001$). Inference drawn from this study is that the OSD is related to anti-glaucoma therapy and has a impact on their quality of life. One should encouraged the use of fixed combinations to reduce surface exposition. A good correlation to signs of OSD was shown by GSS has shown and it should be routinely used to assess the impact of OSD on the quality of life in patients with OSD.

14. Saini M et al[61] evaluated the ocular surface changes and their correlation with the central corneal sub-basal nerve fibre layer in chronic glaucoma patients in a prospective comparative study. Ocular surface assessment was done in 50 eyes of 25 glaucoma patients on combination therapy with two or more topical antiglaucoma medications with preservatives for at least a period of 6months and 50 eyes of 25 normal subjects without any ocular problems were taken as controls. All the enrolled subjects were followed up during the period of November 2011 to November 2013Parameters evaluated in the study were visual acuity, IOP, ocular surface assessment via fluorescein break-up time (FTBUT), Schirmer's test I, ocular surface staining scores and OSDI questionnaire score (OSDI) , central corneal sensation (by Cochet Bonnett aesthesiometer), central sub-basal nerve fiber layer density (SBNFLD) by confocal microscopy. A statistically significant difference were seen in the mean values of OSDI score (35.89±16.07/6.02±3.84; P=0.001), FTBUT (9.44±2.76s/11.8±1.88s; P=0.001), Schirmer's I test score (7.63±2.64 mm/12.86±1.93 mm; P=0.001), corneal (5.7±2.33/ 1.1±0.58; P=0.001) and conjunctival staining score (5.06±1.94/0.84±0.46; P=0.001), mean subbasal nerve fiber number (3.58±0.99/5.40±1.70; P=0.001), SBNFL length (1101.44±287.56 μm/1963.70±562.56 μm; P=0.001) and density

(6883.94±1798.03 μm/mm^2/12 273.15±3516.04 μm/mm^2; P=0.001) when comparison was made between the treated group and controls.66% of glaucoma group showed dry eye severity of level 2 and 3.A statistically significant negative correlation with central corneal SBNFLD was observed with corneal (R2=0.86) and conjunctival staining (R2=0.71) and OSDI score (R2=0.67) but a positive correlation was seen between FTBUT (R2=0.84), corneal sensitivity (R2=0.52) and central corneal SBNFLD in the long term topical antiglaucoma medication group.Long term topical antiglaucoma medications use resulted in reduced SBNFLD and this was also associated with ocular surface changes and antiglaucoma therapy induced dry eye.

15. Another cross-sectional, investigator-masked , paired-eye comparison study to evaluate tear film parameters, ocular surface characteristics, and dry eye symptomology in glaucoma patients on topical anti-glaucoma medications was done by Wong ABC et al.[62] They enrolled 33 patients with a diagnosis of either open angle glaucoma or ocular hypertension, on topical anti-glaucoma medication only in one eye for a period of at least 6 months. All the participants were asked to skip the morning dose of topical anti-glaucoma medications before the clinical evaluation . Assessment and comparison of tear film parameters, ocular surface characteristics, and dry eye symptomology was done in both treated and fellow eyes of the same patient. All the participants underwent evaluation of tear meniscus height, non-invasive tear film breakup time, tear film lipid layer grade, bulbar conjunctival hyperaemia, tear osmolarity, ocular surface staining, meibomian gland expression, infrared meibography , Schirmer I test. OSDI questionnaire was used to grade the Dry eye symptomology. Non-invasive tear film break-up time (p=0.03), bulbar conjunctival hyperaemia (p=0.04), tear film osmolarity (p=0.04), eyelid margin abnormality grade (p=0.01), tear meniscus height (p=0.03), and Schirmer test values (p=0.04) were significantly poorer in treated eyes as compared to fellow eye. They found no significant difference between treated and fellow eyes (all p>0.05) in terms of dry eye symptomology, meibomian gland assessments, and ocular surface staining. Hence this study suggested that longer duration of topical anti-glaucoma therapy sets up an inflammatory cascade which leads to adverse changes in tear film stability, tear osmolarity, conjunctival hyperaemia, and eyelid margin.

16. Lee SY et al[63] evaluated the effects of topical anti-glaucoma medications on tear lipid layer thickness (LLT) and the ocular surface. It was a retrospective study which enrolled 85 eyes of 85 subjects with ocular surface disease (OSD). Controls were 34 eyes who were

OSD patients without OAG and 51 eyes were taken in the group of OSD patients with OAG on topical anti-glaucoma medication. Lipid layer thickness by Lipi- View interferometer, total corneal and conjunctival staining (Oxford grading scale), tear breakup time (TBUT) and Ocular Surface Disease Index (OSDI)questionnaire were used to evaluate OSD in enrolled participants. Information regarding total duration for which anti-glaucoma medication were used, duration for which current anti-glaucoma medications are use, total number of drops of anti-glaucoma medication applied daily and total number of anti-glaucoma medications [bottles] used in a day were gathered to verify their associations with LLT. Among the various parameters assessed ,only LLT values had significantly lower results in OAG group. A significantly negative correlation was seen between LLT and TBUT , in both groups. OSDI score had a significantly positive correlations with number of medications used ($r = 0.389$, $p = 0.012$) and daily number of drops ($r = 0.354$, $p = 0.02$) and similarly LLT showed significant correlations with TBUT ($r = 0.381$, $p = 0.026$) and total medication duration ($r = j0.387$, $p = 0.013$) in the OAG group. TBUT and total medication duration had a significant correlations with LLT ($p = 0.032$ and $p = 0.015$, respectively) in multivariate analyses done in the OAG group. Therefore , OAG subjects with OSD who were on topical anti-glaucoma medications had a lower tear LLT as compared to OSD subjects without OAG. So it is prudent to look for ocular surface disease in glaucoma patients on topical anti-glaucoma medications.

17. Van Went C et al[(64)] conducted an observational study with the purpose to assess the effect of topical anti-glaucoma medications and their preservatives on corneal sensitivity.95 eyes of 48 patients were enrolled in the study. Out of which 39 patients were on anti-glaucoma medications [30 patients had chronic open-angle glaucoma (62.5%), 5 patients had chronic narrow-angle glaucoma (10.4%), 4 patients had ocular hypertension (8.3%)] and 9 untreated patients were taken as controls. Patients on anti-glaucoma medications were divided into three groups depending upon the number of preserved anti-glaucoma eyedrops used daily (0, 1 and ≥ 2). A detailed evaluation of ocular surface including Schirmer testing, tear film breakup time (BUT) and corneal and conjunctival fluorescein staining and assessment of corneal sensitivity by Cochet-Bonnet esthesiometer was done in all the patients. Ocular symptoms were evaluated via Ocular Surface Disease Index (OSDI) questionnaire. Results showed that corneal sensitivity was 58.8 ± 2.8 mm, 56.2 ± 5.2 mm, 50.3 ± 12.5 mm and 44.3 ± 13.6 mm in untreated patients, patients treated with none, one and two or more instillations of preserved anti-glaucoma eyedrops, respectively. A significantly

lower corneal sensitivity was noted in patients on preserved eyedrops as compared to untreated patients (P < 0.001) and those on preservative-free eyedrops (P = 0.012).Negative correlations were observed between number of daily instillations of eyedrops (r = –0.390 ; P < 0.001) , duration of treatment (R = –0.357 ; P = 0.001) with corneal sensitivity . A significant alteration in TBUT and fluorescein staining were in treated patients as compared to untreated control group ; but , no significant difference was seen among the treated groups. There was no significant difference for OSDI or Schirmer testing between the different groups. Conclusion deduced from this study was that the Chronic administration of BAK-containing anti-glaucoma eyedrops leads to alteration in corneal sensitivity.

18. Brandt JD et al[37] conducted a study in 1991.In this study they used the technique of conjunctival impression cytology to evaluate the conjunctival surface changes that results from long-term use of topically administered anti- glaucoma medications. Glaucoma patients who were on a stable regimen of topically administered anti-glaucoma medications for more than three months duration were recruited as cases while glaucoma suspects who were not on any topical anti-glaucoma medications were taken as controls. Conjunctival impression cytology specimens were collected from the bulbar and palpebral conjunctiva. Average scores from the four bulbar conjunctiva were compiled and graded according to by Nelson grading with a range of 0 (normal) to 3 (diffuse, severe metaplasia).A statistically significant degrees of conjunctival metaplasia was associated with the number of glaucoma medications used. Therefore conjunctival surface changes were present in patients on longer duration of topical anti-glaucoma medications use.

19. Baffa L do P et al[65] carried out a study to assess whether chronic use of topical anti-glaucoma medications can induce changes in tear film and ocular surface or not. The study included 21 glaucoma patients on topical anti- glaucoma medications for more than 8 months as cases and 20 age- and sex- matched volunteers without any eye and systemic medications as control group. All the participants filled environmental questionnaire with 10 questions and score range from 0 to 6, from absent to severe discomfort. Tear film and ocular surface examination was done by tear film break-up time (TFBUT), 2% fluorescein and 1% lissamine green staining, Schirmer test and conjunctival impression cytology [specimens were classified into four stages, stage 0 (normal morphology) to stage 3 (squamous metaplasia)].The study found that significantly higher fluorescein staining (p=0.003), lissamine green staining (p=0.02) and lower TFBUT (p=0.001)values were

present in glaucoma patients with chronic use of topical anti-glaucoma medications when compared to controls but no difference was seen in impression cytology results between the cases and control group ($p>0.05$).The study concluded that chronic use of topical anti-glaucoma medications leads to changes in tear film and ocular surface.

20. A prospective study was done by Jandroković S et al[66] to calculate the prevalence of ocular surface disease symptoms and the status of the tear film in glaucoma patients on topical intraocular pressure lowering monotherapy. The study enrolled 62 patients out of which 32 participants had primary open-angle glaucoma in both eyes and were on one topical antiglaucoma medication for ≥ 6 months and 30 healthy participants were included as controls. They performed tear film break-up time (TBUT) test , Schirmer test type1 and ocular surface disease questionnaire in both groups. Abnormal values of tear break-up time (TBUT) were higher in treatment group in comparison to control group. Advanced disorder in TBUT was present in seven (21.9%) glaucoma patients and none of the control group participants. Schirmer test type 1 found that the basal tear secretion was reduced in 87% of glaucoma patients and in 16.7% control group participants. Hence, glaucoma patients on topical anti-glaucoma monotherapy had significantly higher prevalence of ocular surface disease symptoms along with significant reduction in basal tear secretion and destabilisation of tear film when compared to healthy volunteers.

21. Herreras JM et al[67] conducted a study to see whether long term topically applied anti-glaucoma medications produces any changes in ocular surface and particularly any damage is caused to the mucus layer of tear film or not. Three groups were studied. Group 1consisted of 40 subjects taken as controls. Group 2 included 21 glaucoma patients treated with 0.5% timolol maleate for a mean of 25.1 months and group 3 enrolled 20 untreated glaucoma patients (evaluated before starting treatment and then monthly for 3 months). In all the participants Schirmer's test, Tear meniscus height, Tear break-up time, fluorescein and rose Bengal staining, conjunctival impression cytology, mucus staining, and ferning test were performed. This study showed that as compared to subjects in group 1, the subjects in groups 2 and 3 showed a significant decrease ($P < 0.001$) in the values Schirmer's and break-up time tests, positive vital stains with significant reduction ($P < 0.001$) in goblet-cell density, mucus granules, and reticular sheets, and an increment ($P < 0.001$) in pathologic crystallization patterns. Therefore, the chronic use of a topical timolol maleate (anti-glaucoma medication) damages the ocular surface, especially the mucus layer of the tear film.

22. Yalvaç IS et al[68]carried out a study to see the effects of topical antiglaucoma medications on ocular surface. The effects were analyzed by performing Schirmer's test, tear break-up time test and conjunctival impression cytology. 20 healthy subjects as controls (group I), 20 POAG patients (group 2) treated with 0.50% timolol maleate (containing BAK) and 20 POAG patients (group 3) treated with 0.50% timolol maleate +1% dipivefrin hydrochloride (containing BAK) were enrolled in the study. Schirmer's test and tear film break-up time values were significantly reduced in group 2 and 3 as compared to group 1 ($p<0.001$).In conjunctival impression cytology also there was a significant degree of squamous metaplasia in group 2 and 3 participants when compared to controls along with a significantly reduced goblet cell density in group 2 and 3.Results of above study were suggestive of damaging effects of topical anti-glaucoma medications on ocular surface.

23. Arici MK et al[69] assessed the effects of topical anti-glaucoma medications on ocular surface when used for longer duration of time. The study evaluated the ocular surfaces of 30 healthy controls (group 1) and 77 POAG patients (on topical anti-glaucoma drugs for at-least 2 years) by performing basal Schirmer's test, tear film break-up time test and conjunctival impression cytology. 24 patients were on 0.5% betaxolol hydrochloride (group 2) , 27 patients on 0.5% timolol maleate (group 3) and 26 patients on 0.5% betaxolol and 1% dipivefrin hydrochloride (group 4).The study found the statistically significant reduction in values of basal Schirmer's tests and tear break-up time tests ($P < 0.01$) in groups 2, 3 and 4. Conjunctival impression cytology scores were also significantly higher in groups 2, 3 and 4 than in group 1 ($P < 0.01$).Hence it is possible to have conjunctival surface and tear film function changes after the long-term use of topical antiglaucoma medication.

24. Cvenkel B et al[70] conducted a cross-sectional, case-comparison study to evaluate the signs and symptoms of ocular surface disease (OSD) and cytomorphological changes in ocular surface due to preserved topical anti-glaucoma medications. The study included 109 participants, out of which 79 were glaucoma patients on preserved topical anti-glaucoma drugs for at least 6 months duration and 30 (control group) were patients with ocular hypertension and relatives of glaucoma patients who were not on any topical antiglaucoma drugs. Ocular Surface Diseases Index (OSDI) questionnaire was filled by all the participants. Beside OSDI) questionnaire, Schirmer test, tear film breakup time (TBUT), fluorescein staining and conjunctival impression cytology were done. Patients on topical antiglaucoma medications had reduced TBUT, an increase in fluorescein staining grade along with

increased impression cytology grade as compared to the control group. Although the OSDI questionnaire score didn't differ statistically between the two groups. More the number of eye drops instilled per day in the treated group , higher the grade of fluorescein staining ($P < 0.001$) and a shorter TBUT. But the impression cytology grades were not statistically significantly correlated with the number of topical anti-glaucoma medications used. So, the study concluded that the preserved topical anti-glaucoma medications can damage the ocular surface.

MATERIALS & METHODS

MATERIALS AND METHODS

The study was conducted in the Department of Ophthalmology, in collaboration with the

Department of Community Medicine and Family Medicine and Department of Pathology & Lab Medicine AIIMS, Jodhpur.

Study Design: Prospective Cohort Study

Inclusion Criteria	Exclusion criteria
1. Consent to inclusion and participation in study. Informed and written consent was obtained from all the subjects included in the study. 2. Newly diagnosed cases of adult primary open angle glaucoma not on any topical Anti-glaucoma medications 3. Adult patients of primary open angle glaucoma on topical Anti-glaucoma medications	1.Those subjects who have undergone cataract surgery/glaucoma surgery/laser procedure within last 6months (to avoid cases of OSD due to post-surgery eyedrops) 2.Those with ocular conditions that can contribute to the occurrence of dry eye, such as lid disorders (blepharitis, ectropion, entropion), contact lens wear, allergic conjunctivitis, chronic conjunctivitis, exposure keratitis, contact dermatitis, and Bell's palsy; those with systemic conditions like, thyroid, diabetes, lupus, rheumatoid arthritis, scleroderma, Sjögren's syndrome, vitamin A deficiency, and other factors like smoking; and those with continuous long-term use of ocular or systemic medications (antihistaminic, antidepressants, decongestants, beta blocker drugs, diuretics, and aspirin),disorders decreasing corneal sensitivity will be excluded 3. Subjects not giving written informed consent 4. Uncooperative patients in whom the parameters cannot be measured.

SAMPLE SIZE: In the study by Saini et al. conducted in 2017, various parameters were studied to evaluate the ocular surface. Among them, the mean values of Fluorescein Tear-film Breakup Time (FTBUT) between two groups have been used to calculate the sample size.

At 99% level of confidence and 90% power and an allocation ratio of 1, the sample size had been calculated as 60 eyes in each group (based on the difference between the means). Therefore, the total numbers of 120 eyes were recruited in the study.

Methodology

The study was conducted in the Department of Ophthalmology, All India Institute of Medical Sciences, Jodhpur in collaboration with the Department of Community Medicine and Family Medicine and Department of Pathology and Lab Medicine, All India Institute of Medical Sciences, Jodhpur after obtaining approval by Institutional Ethics Committee (IEC AIIMS/IC/2021/3351), AIIMS Jodhpur. Our study was adhered to declaration of Helsinki. Study included all the adult primary open angle glaucoma patients who visited to the glaucoma clinic of eye department, AIIMS Jodhpur between January 2021 to June 2022.All the enrolled patients underwent comprehensive glaucoma evaluations along with ocular surface examination at day 1,1 mont,3 months, 6 months and 12 months follow up.

The diagnosis of primary open angle glaucoma was made on the basis of characteristic disc findings, gonioscopic features and visual field changes. A written informed consent was taken and a Patient Information Sheet was provided to them.

All the patients included in the study were divided into 2 groups-

1. Newly diagnosed patients of primary open angle glaucoma not on any treatment
2. Patients of primary open angle glaucoma on topical anti-glaucoma medications.

In all the participating patients, a detailed history regarding onset of symptoms, if any, duration, progression and any associated complaints, history of any medical illness or addiction, family history of glaucoma, thorough treatment history was taken. After ensuring that the patient satisfied our inclusion criteria, the patient was informed regarding the nature of study and each patient was given a patient information sheet. Patient was enrolled in the study after obtaining informed written consent. The demographic details of all the participating patients were recorded.

All the participants included in the study, filled the Ocular Surface Disease Index (OSDI) questionnaire. The baseline parameters including visual acuity (both unaided and best corrected) using Snellen's Charts, intraocular pressure (IOP) with Goldmann applanation tonometer, central corneal thickness with the help of autorefractometer and detailed slit-lamp examination, visual fields assessment, dilated fundus examination with +90D lens and indirect ophthalmoscopy with +20 D lens were performed in each patient.

Figure 12: Measurement of Central Corneal Thickness Using an Autorefractometer (Source: Department of Ophthalmology, AIIMS Jodhpur)

Figure 13: Intraocular pressure measurement by Goldmann applanation tonometer (Source: Department of Ophthalmology, AIIMS Jodhpur)

In addition, tear break-up time (TBUT) test, Schirmer's test (type I), corneal sensitivity, anterior segment-optical coherence tomography, staining of the cornea and conjunctiva and conjunctival impression cytology was carried out in all the participants for evaluation of ocular surface disease.

The diagnosis of Ocular surface Disease was based on:

OSDI questionnaire, (developed by Allergan)[8][9] is a disease-specific questionnaire used to quantify the specific impact of dry eye on vision-related quality of life. It includes 12 questions that are divided into three parts: questions 1–5 refer to ocular pain or visual difficulties such as blurred vision or light sensitivity; questions 6–9 is about visual functionality and questions the ability to read or drive at night; and questions 10–12 analyze environmental factors such as air conditioning or wind. The responses range from 0 to 4 with 0 indicating none of the time and 4 indicating always; the OSDI score range from 0 to 100. The criteria used for the grading was: 0-12= normal; 13-22= mild; 23-32= moderate; and 33-100= severe.

The total OSDI score was calculated by using the formula:

$$\text{OSDI} = \frac{\text{Sum of scores for all the questions asked}}{\text{Total number of questions answered}} \times 25$$

Schirmer's test type 1 was done to test basal and reflex tear secretion by using a Schirmer's strip prepared from Whatman filter paper no. 41, measuring 40×5 mm, marked 0 to 35 mm. 5 mm at the top end of a filter paper strip was folded and the patient was asked to look up and with an index finger, lower eyelid was gently pulled down and filter paper strip was placed into the cul-de-sac at the junction of lateral one-third and medial two-thirds of the lower lid. The patient was instructed to gently close the eyes and the strip was removed at 5 minutes and the extent of wetting of the strip was measured. Depending on the wetting of the strip after 5min, the results of Schirmer's test were graded as: >10 mm= normal (grade 0); 5-10 mm= mild (grade 1); 3-4 mm= moderate (grade 2); 0-2 mm= severe (grade 3).

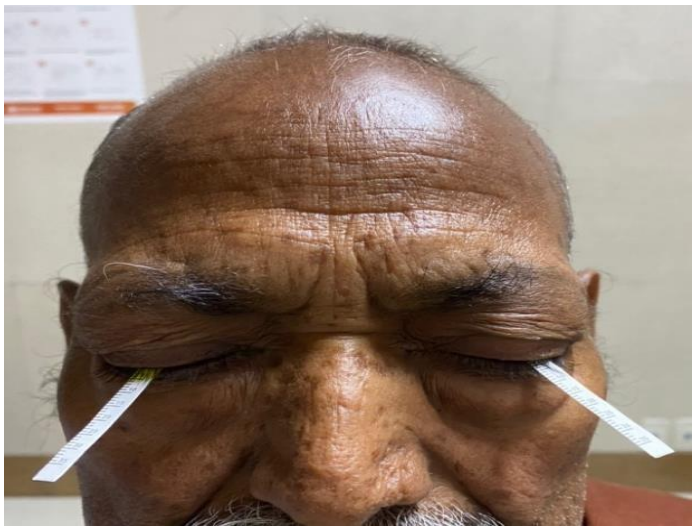

Figure 14: Schirmer's test type 1

(Source: Department of Ophthalmology, AIIMS Jodhpur)

Tear film break-up time (TBUT) test is a method of determining the stability of the tear film. TBUT is the time interval between the last blink and tear film disruption. One drop of topical anesthetic agent was instilled in the eye and this was followed by application of fluorescein dye into the inferior palpebral conjunctiva after gentle depression of the lower eyelid and then the patient was asked to blink several times for even spread of fluorescein dye. Examination of the tear film was done on slit lamp with cobalt blue filter. The interval between the last blink and the appearance of a first hypo-fluorescent spot or streak was recorded as the TBUT. TBUT less than 10 seconds is abnormal and graded as: >10 seconds = normal (grade 0);6.1-10 seconds = fair (grade 1); 3.1-6 seconds = moderate (grade 2); <3 seconds = poor (grade 3)

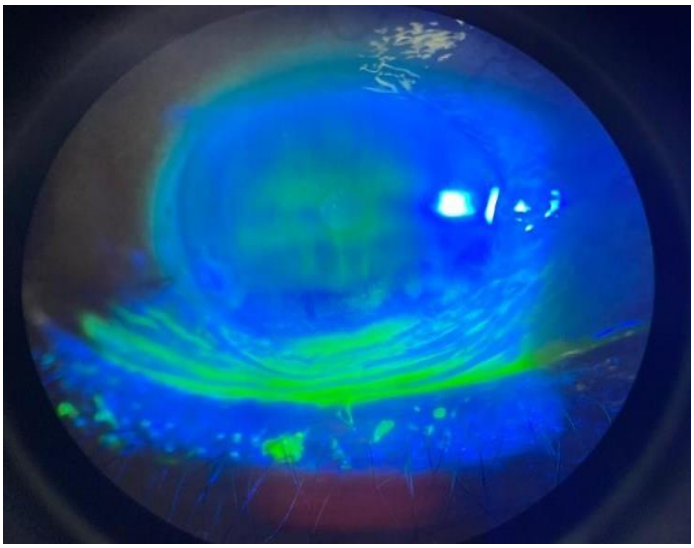

Figure 15: Tear film break-up time test under cobalt blue filter
(Source: Department of Ophthalmology, AIIMS Jodhpur)

After explaining the procedure to the patient, the patient was asked to view at a distance object and then the cotton wisp was brought from the side and the central cornea was touched gently with the tip of cotton wisp. The presence of blink reflex was observed. Corneal sensations were checked in all the quadrants in a similar manner.

Anterior segment- optical coherence tomography was done to assess lower tear meniscus height (L-TMH), lower tear meniscus depth (L-TMD) and lower tear meniscus area (L-TMA). Tear meniscus appears as triangular shape of wedge of tear film between the lower lid margin and ocular surface. After obtaining the tear meniscus images, the L-TMH ,L-TMD and TMA were calculated using the ImageJ software (Java software program developed by the National Institutes of Health, Bethesda, MD; http://imagej.nih.gov/ij/). TMH was measured for 3 times in each photograph, and the mean value was recorded for further analysis. The scan was taken below the corneal vertex, centered on the inferior cornea and lower eyelid.[54] Corneal vertex was determined by the position of specular reflection.[54] The subject was asked to blink normally during the imaging procedure while looking straight at a fixed target within the device. Images were captured within the first second immediately after a blink.

Figure 16: Anterior segment- optical coherence tomography of tear meniscus
(Source: Department of Ophthalmology, AIIMS Jodhpur)

Figure 17: Lower tear meniscus on AS-OCT
(Source: Department of Ophthalmology, AIIMS Jodhpur)

Conjunctival and corneal staining was done by using sterile rose bengal paper strips and lissamine green paper strips. The strip was applied for 2 minutes in lower outer conjunctional cul-de-sac. Then the patient was asked to blink multiple times for uniform spread of the dye. Examination was done on slit lamp biomicroscope under white light. Ocular surface damage was graded by using the Oxford grading scheme. The grading chart consisted of a series of panels, labelled A–E, in increasing order of severity, denoting the staining patterns seen in

dry eye. These panels were used as a guide to grade the degree of staining seen in the patient. Amount of staining was represented by punctate dots.

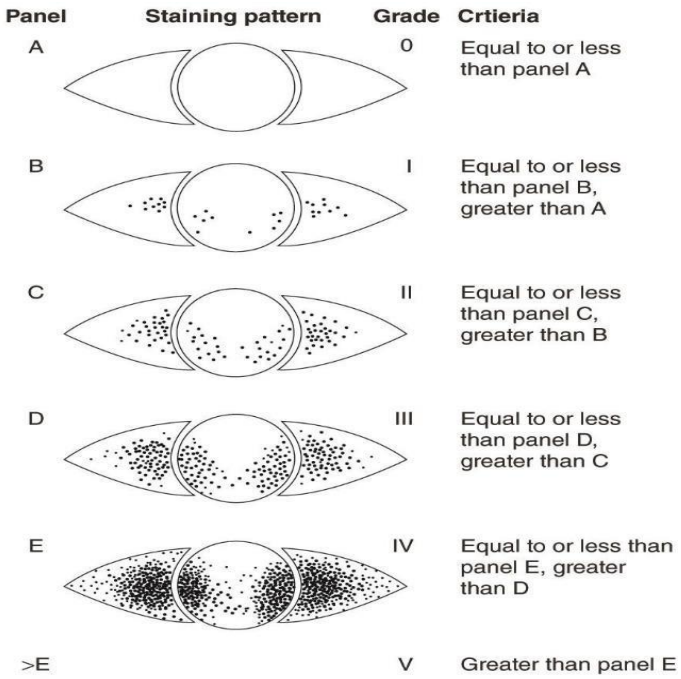

Figure 18: Oxford grading scale

(Adapted from https://images.app.goo.gl/krNApxF8BrEYut1q6)

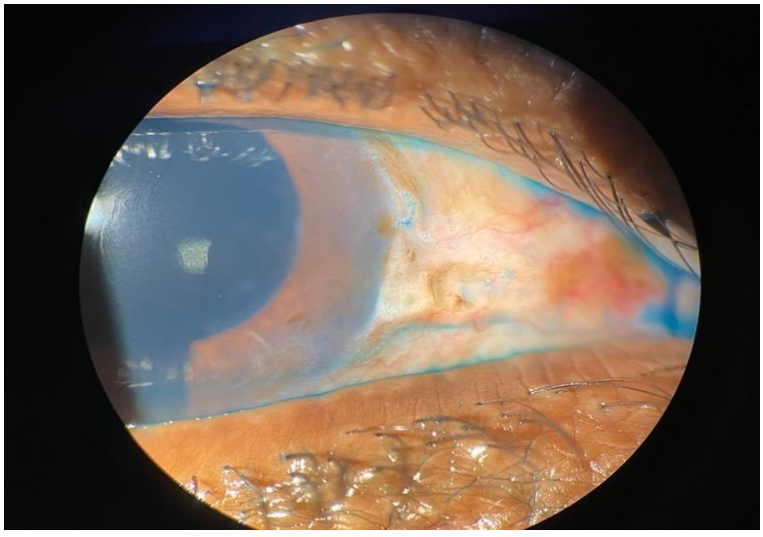

Figure 19: Lissamine green staining of ocular surface

(Source: Department of Ophthalmology, AIIMS Jodhpur)

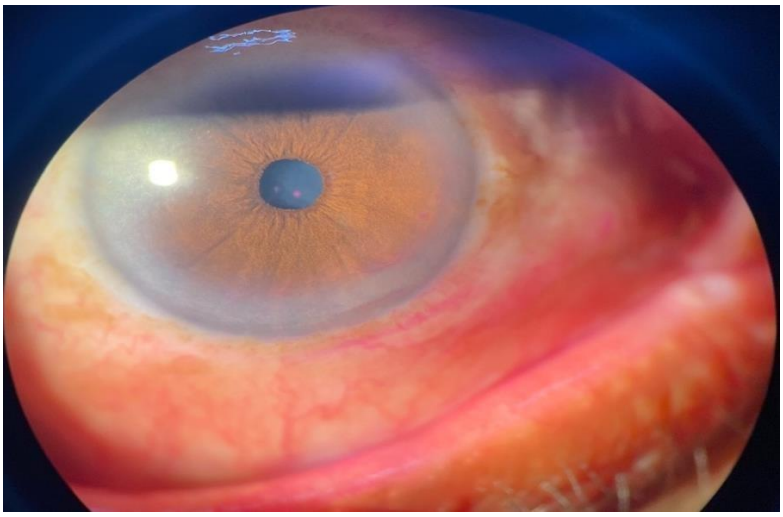

Figure 20: Rose Bengal staining of ocular surface
(Source: Department of Ophthalmology, AIIMS Jodhpur)

After using all aseptic precautions, the conjunctival impression smear was taken on 1x1 cm strip of cellulose acetate filter (micropore size) paper after applying it to upper temporal bulbar conjunctiva of the eye. The strip was kept firmly pressed for five seconds. Two smears were taken from each eye. Strip was then transferred immediately on to a glass slide and fixed immediately using 95% ethanol. Then the smears were transferred to the department of pathology and lab medicine, AIIMS jodhpur where one of the smears were fixed and subjected to Papanicolau stain and the other to Periodic Acid Schiff stain. The stained smears were studied and graded by an experienced pathologist (blind observer) into Goblet cells density of >75/HPF, 50-75/HPF, 15-50/HPF, <15/HPF; mild, moderate and severe squamous metaplasia and presence or absence of inflammatory cells.

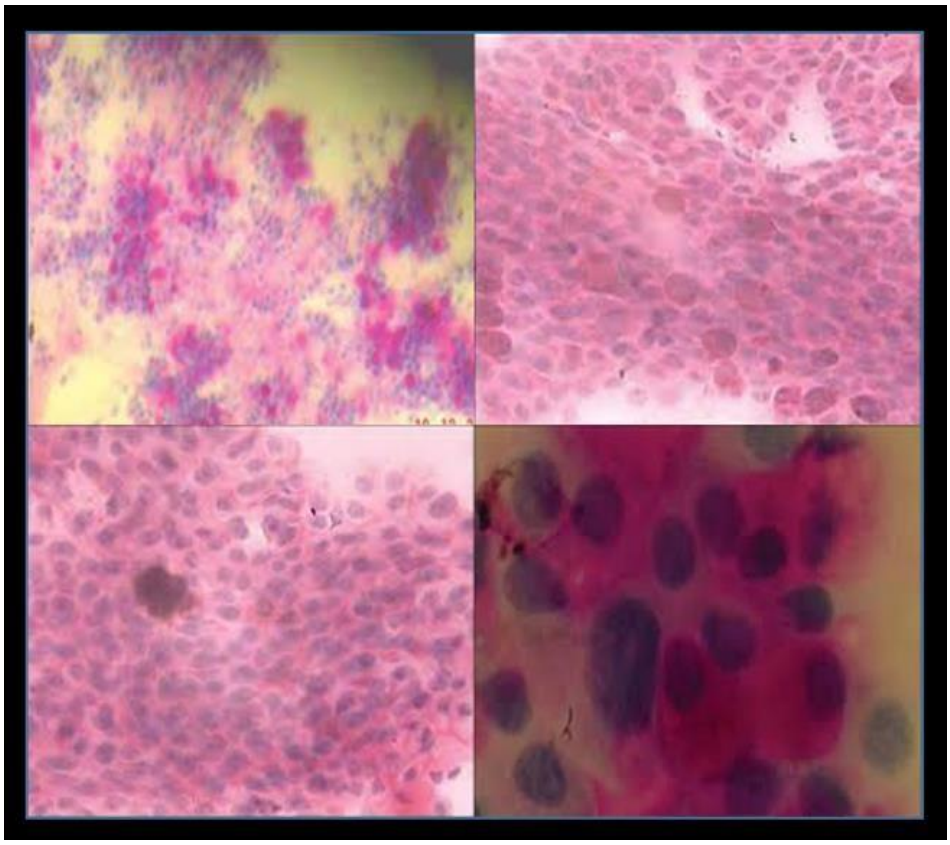

Figure 21: Impression cytology smear
(Source: Department of Pathology and Lab Medicine, AIIMS Jodhpur)

STATISTICAL ANALYSIS

- Data was entered and analysed using the SPSS (Statistical Package for the Social Sciences) version 25. SPSS Statistics is a statistical analysis software package that allows you to perform interactive or batch statistical analysis. On August 8, 2017, SPSS Statistics released version 25. It was long created by SPSS Inc. before being acquired by IBM in 2009. IBM SPSS Statistics is the brand name for current versions (after 2015).
- The nominal, ordinal and continuous variables were identified.
- The data was cleaned and coded for analysis.
- All nominal variables were described using counts and percentages and analysed using Chi Square test or Fischer's Exact test.
- All ordinal variables were described using median and IQR and analysed using Mann Whitney U test.
- All continuous variables were described using mean and SD and analysed using Independent Sample t test.
- The analysed data was organized in tables.
- Graphs were plotted wherever necessary.
- A p value of less than 0.05 was considered statistically significant.

ETHICAL CONSIDERATION

- The following study was conducted after approval from the Institutional Ethics Committee.
- Informed consent was taken from all the patients being enrolled for the study by providing them a proper printed consent form along with patient information sheet and after properly explaining the purpose.

RESULTS

RESULTS

Sixty eyes of 30 newly diagnosed, treatment naive POAG patients (group I) and 60 eyes of 30 POAG patients which were already on topical anti-glaucoma medications (group II) were enrolled. There were 18 males and 12 females in group I and 20 males and 10 female in group II (p=1.00). In group II, 12 eyes were on 1 drop , 18 eyes on 2 drops and 30 eyes on ≥ 3 drops. On further subgroup analysis in group II, 24 eyes were on topical anti-glaucoma medications for < 3 years and 36 eyes were on topical anti-glaucoma medications ≥ 3 years.

Table 1: Age distribution in group I and group II

Age (Years)	Group 1 n (%)	Group 2 n (%)
40-50	20 (33.33%)	8 (13.33%)
51-60	8 (13.33%)	17 (28.33%)
61-70	22 (36.67%)	25 (41.67%)
71-80	10 (16.67%)	10 (16.67%)
Mean ± SD	59.00 ±11.52	60.3±9.31
p value	0.327	

The mean age was comparable in both the groups (Table 1).

Table 2: Baseline characteristics in group I and group II

	Group I	Group II	p value
Visual acuity	0.344±0.618	0.59 ± 0.998	0.107
Baseline IOP	19.66 ± 7.21	17.13 ± 6.209	0.0416
CDR	0.71±0.164	0.733±0.176	0.46
Mean deviation	5.899±2.69	6.64 ± 2.41	0.114

(IOP= Intraocular pressure; CDR= Cup disc ratio)

Mean visual acuity, cup-disc ratio and mean deviation on HVF was comparable in both the groups. Mean IOP was significantly higher in group I (19.66 ± 7.21) in comparison to group II (17.13 ± 6.209),(p=0.0416), (Table 2).

Table 3: Ocular surface parameters of group I and II

Ocular surface parameters	Group I (Mean±SD)	Group II (Mean±SD)	p value
Schirmer's test	20.2±3.02	13.91±3.03	<0.0001
TBUT	12.1±2.31	8.5±1.72	<0.0001
OSDI Questionnaire score	34.25±9.02	35.17±5.98	0.511

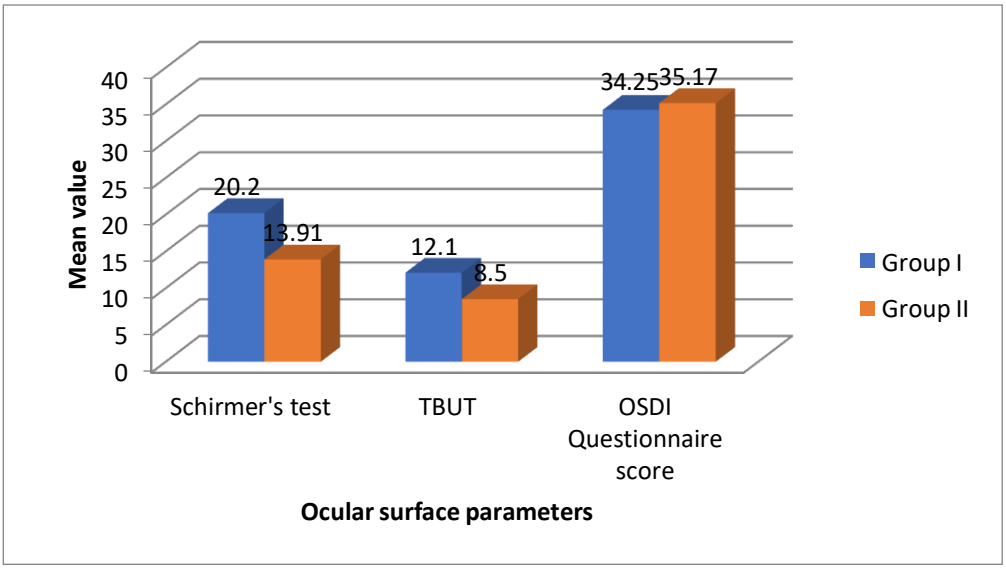

Figure 22: Graphical representation of ocular surface parameters between group I and II

The mean values of Schirmer's test and TBUT at baseline was significantly more in group II than in group I (p <0.0001).There was no significant difference in the mean values of OSDI score at baseline between the groups (p = 0.511), (Table 3) (Figure 22).

Table 4: Baseline Corneal sensations in group I and II

Corneal sensations	Group I n (%)	Group II n (%)
Intact	60 (100 %)	59 (98.33%)
Reduced	0	1 (1.67%)

There was no significant difference in corneal sensations between the two groups on presentation (Table 4).

Table 5: Conjunctival and Corneal Staining at baseline in both the groups

Staining	Grade	Group I n (%)	Group II n (%)	p value
Lissamine green	0	56 (93.33%)	22 (36.67%)	<0.0001
	1	4 (6.67%)	34 (56.67%)	
	2	0	4 (6.67%)	
	3	0	0	
	4	0	0	
	5	0	0	
Rose Bengal	0	48 (80%)	13 (21.67%)	<0.0001
	1	12 (20%)	37 (61.67%)	
	2	0	10 (16.67%)	
	3	0	0	
	4	0	0	
	5	0	0	

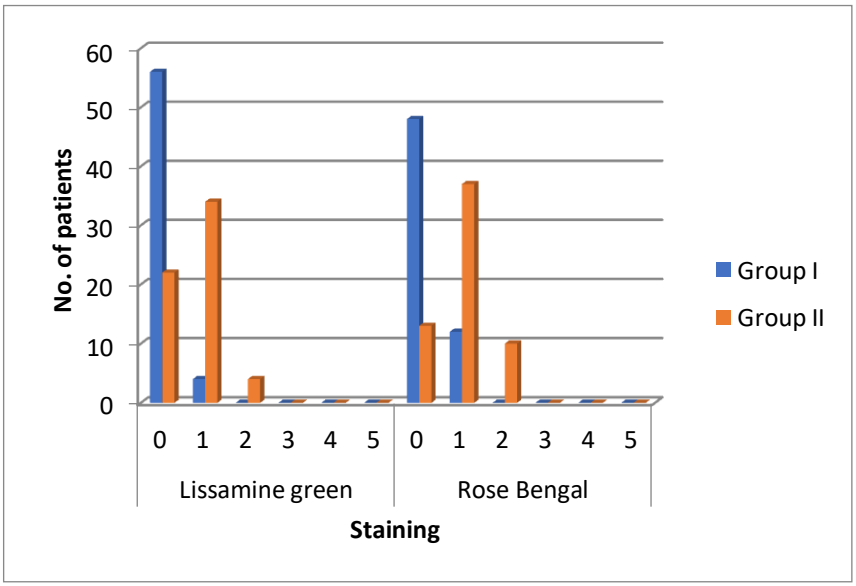

Figure 23: Graphical representation of conjunctival and corneal staining at baseline in both the groups

A significant difference in staining pattern was noted between the 2 groups at baseline (p <0.0001). In group I, 56 (93.33%) eyes showed grade 0,4 (6.67%) eyes showed grade 1 with lissamine green staining and 48 (80%) eyes showed grade 0,12 (20%) eyes showed grade 1 with rose bengal staining. In group II, 22(36.67%) eyes showed grade 0, 34 (56.67%) eyes showed grade 1,4 (6.67%) eyes showed grade 2 with lissamine green staining and 13(21.67%) of eyes showed grade 0, 37(61.67%) eyes showed grade 1 and 10(16.67%) eyes showed grade 2 with rose bengal staining, (Table 5),(Figure 23).

Table 6: Baseline Tear meniscus parameters on AS-OCT in both the groups

Tear meniscus Parameters	Group I (Mean±SD)	Group II (Mean±SD)	p value
TMH-(microns)	253.33±8.86	248.93±6.95	0.001
TMD-(microns)	154.05±5.85	149.9±6.30	0.0003
TMA-(mm2)	0.025±0.029	0.018±0.001	0.099

(AS-OCT- Anterior segment-Optical coherence tomography; TMH-Tear meniscus height; TMD-Tear meniscus depth; TMA-Tear meniscus area)

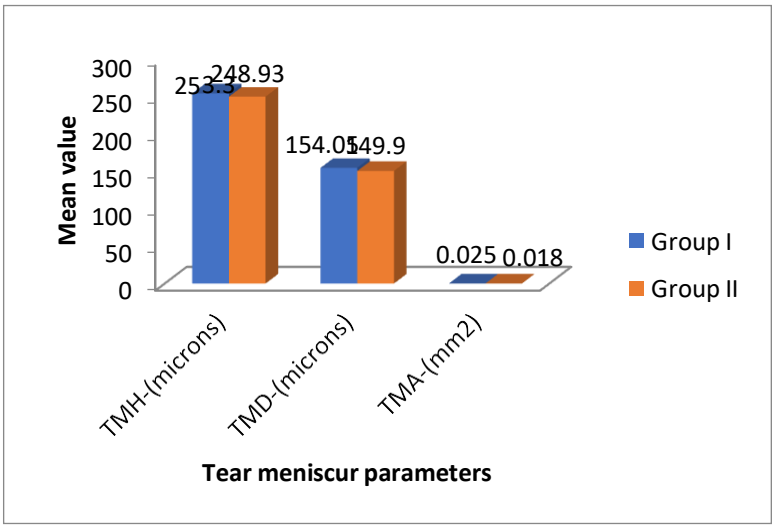

Figure 24: Graphical representation of baseline tear meniscus parameters in both the groups

The mean values of baseline tear meniscus height (253.33±8.86µ vs 248.93±6.95µ) and depth (154.05±5.85µ vs 149.9±6.30µ) on AS-OCT were significantly different between the two groups (p=0.001 and p=0.0003, respectively). There was no significant difference in the mean value of tear meniscus area on AS-OCT at baseline between the two groups (p=0.099), (Table 6),(Figure 24).

Table 7: Conjunctival impression cytology at baseline in both the groups

Conjunctival impression cytology		Group I n (%)	Group II n (%)	P value
Goblet cell density (cells/hpf)	>75	56 (93.33%)	3(5%)	<0.0001
	50-75	4 (6.67%)	24(40%)	
	15-50	0	14 (23.33%)	
	<15	0	19(31.67%)	
Squamous metaplasia	Mild	4 (6.67%)	21(35%)	<0.0001
	Moderate	0	23 (38.33%)	
	Severe	0	16(26.67%)	
	No change	56 (93.33%)	0	
Inflammatory cells	Present	0	53(88.33%)	<0.0001
	Absent	60 (100%)	7 (11.67%)	

(hpf -high power field)

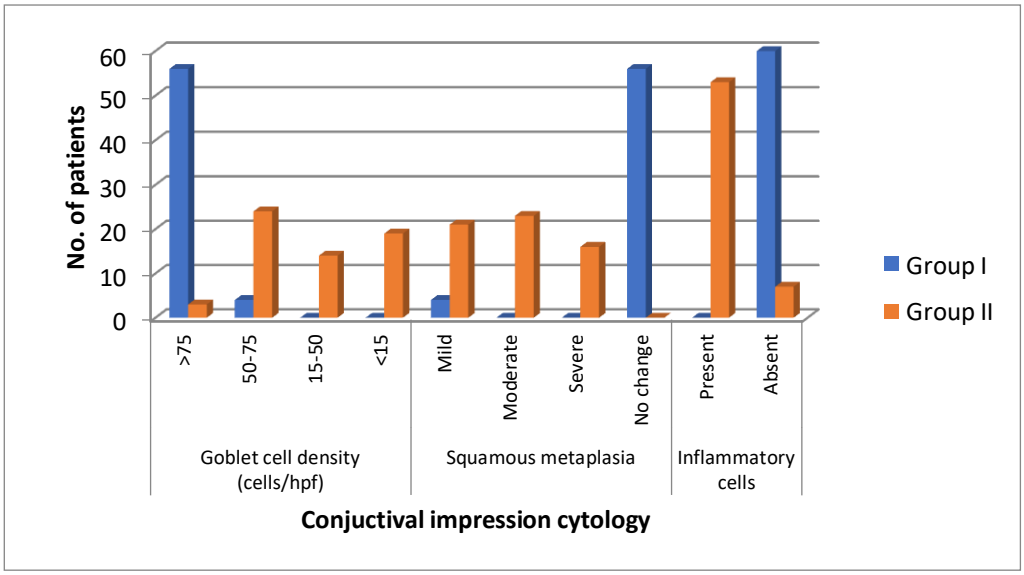

Figure 25: Graphical representation of conjunctival impression cytology at baseline in both the groups

On impression cytology, goblet cell density (cells/hpf) was significantly less in group 1 as compared to group 2 (p <0.0001) at baseline. In group I, 56(93.99%) of eyes had >75cells/hpf, 4(6.67%) of eyes had density between 50-75 cells/hpf. In group II, 3 (5%) eyes

had >75cells/hpf, 24(40%) eyes had density between 50-75 cells/hpf,14 (23.33%) eyes had density between 15-50 cells/hpf ,while 19(31.67%) eyes had density <15 cells/hpf. Additionally, there was a significant amount of squamous metaplasia in group 2 as compared to group 1 (p <0.0001). In group I, 4(6.67%) eyes had mild squamous metaplasia while 56(93.99%) eyes showed no squamous metaplasia. In group II,21(35%) eyes had mild ,23(38.33%) had moderate and 16(26.67%) had severe squamous metaplasia. In group I , none of the impression cytology specimens showed the presence of inflammatory cells while inflammatory cells were present in 88.33% of specimens in group II (Table 7),(Figure 25).

Table 8: Changes in Ocular surface parameters in group I on follow up

Parameters	Baseline	1 month	3 months	6 months	12 months	p value
OSDI Questionnaire score	34.25±9.02	35.23±8.55	36.81±9.08	38.60±7.98	39.46±7.64	0.0009
Schirmer's test	20.20±3.02	18.77±2.48	17.23±2.37	15.73±1.84	14.25±1.89	<0.0001
TBUT	12.10±2.31	10.92±1.90	9.97±2.11	8.55±1.56	7.72±1.29	<0.0001

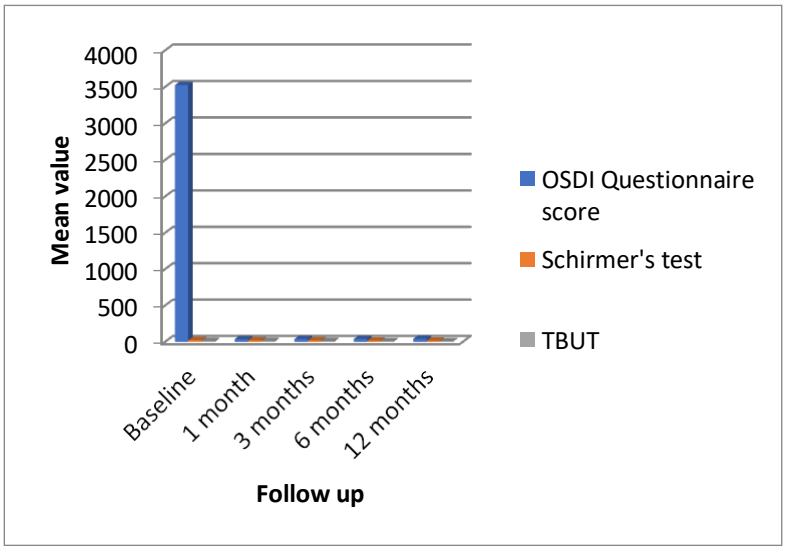

Figure 26: Graphical representation of ocular surface parameters in group I on follow up

In group I, the mean values of schirmer's test and TBUT decreased significantly ($p < 0.0001$) at each follow up visit from baseline. Mean OSDI score increased significantly at 6 month ($p=0.006$) and 12 months ($p=0.0009$) follow up visit when compared to baseline mean values (Table 8),(Figure 26).

Table 9: Changes in corneal sensitivity in group I on follow up

Corneal sensitivity	Follow up				
	Baseline	1 month	3 months	6 months	12 months
	n (%)	n (%)	n (%)	n (%)	n (%)
Intact all quadrant	60 (100%)	60 (100%)	60 (100%)	60 (100%)	60 (100%)
Reduced	0	0	0	0	0

No change in corneal sensitivity was seen on follow up as compared to baseline,(Table 9).

Table 10: Changes in tear meniscus parameters on AS-OCT on follow up in group I

Tear meniscus parameters	Baseline (Mean±SD)	1 month (Mean±SD)	3 months (Mean±SD)	6 months (Mean±SD)	12 months (Mean±SD)	p value
TMH	253.68±8.87	253.22±9.12	250.82±9.06	247.03±8.97	243.52±8.96	< 0.0001
TMD	154.05±5.85	153.33±5.84	151.13±5.99	148.02±6.14	144.53±6.33	< 0.0001
TMA	0.025±0.029	0.019±0.001	0.018±0.001	0.018±0.001	0.017±0.001	0.0348

(AS-OCT- Anterior segment-Optical coherence tomography; TMH-Tear meniscus height; TMD-Tear meniscus depth; TMA-Tear meniscus area)

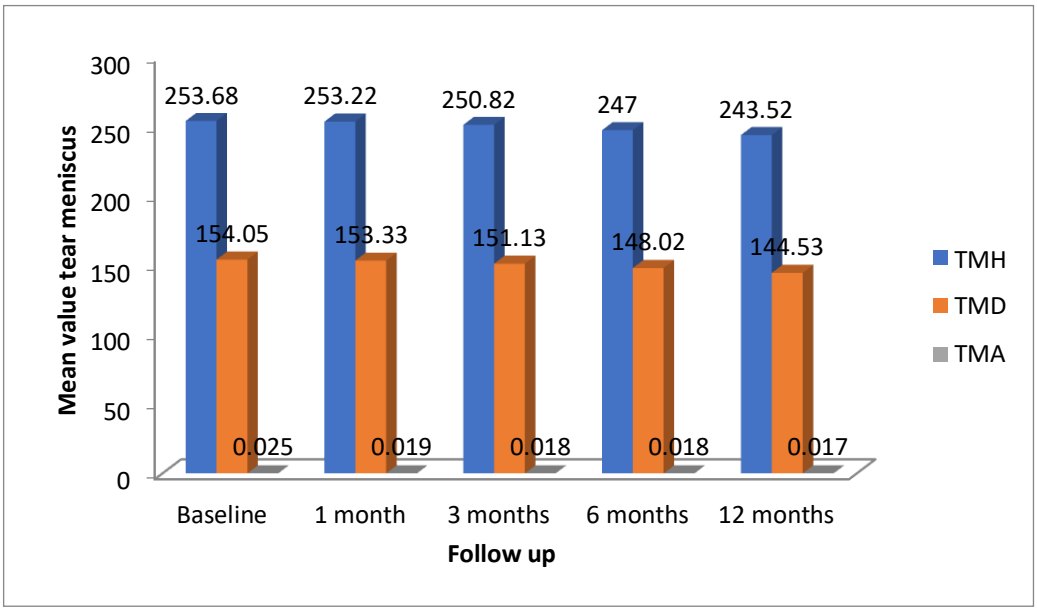

Figure 27: Graphical representation of tear meniscus parameters on AS-OCT on follow up in group I

In group I, the mean values of tear meniscus height decreased significantly at 6 months (p=0.0001) and at 12 months (p<0.0001) follow up visit as compared to baseline values. The mean values of tear meniscus depth decreased significantly at 3month(p=0.0079) at 6 months(p<0.0001) and at 12 months (p<0.0001) follow up from baseline. Although, the

mean value of tear meniscus area reduced on follow up visits but the difference was not statistically significant (P>0.05), (Table 10),(Figure 27).

Table 11: Changes in conjunctival and corneal staining in group I on follow up

Staining	Grade	Follow up				
		Baseline	1 month	3 months	6 months	12 months
		n (%)	n (%)	n (%)	n (%)	n (%)
Lissamine green	0	56 (93.34%)	55 (91.66%)	52 (86.666%)	43 (71.66%)	24 (40%)
	I	4 (6.66%)	5 (8.33%)	8 (13.33%)	17 (28.33%)	30 (50%)
	II	0	0	0	0	6 (10%)
	II	0	0	0	0	0
	IV	0	0	0	0	0
	V	0	0	0	0	0
Rose bengal	0	48 (80%)	46 (76.66%)	41 (68.88%)	37 (61.66%)	14 (23.33%)
	I	12 (20%)	14 (23.33%)	19 (31.66%)	21 (35%)	35 (58.33%)
	II	0	0	0	2 (3.33%)	11 (18.33%)
	II	0	0	0	0	0
	IV	0	0	0	0	0
	V	0	0	0	0	0

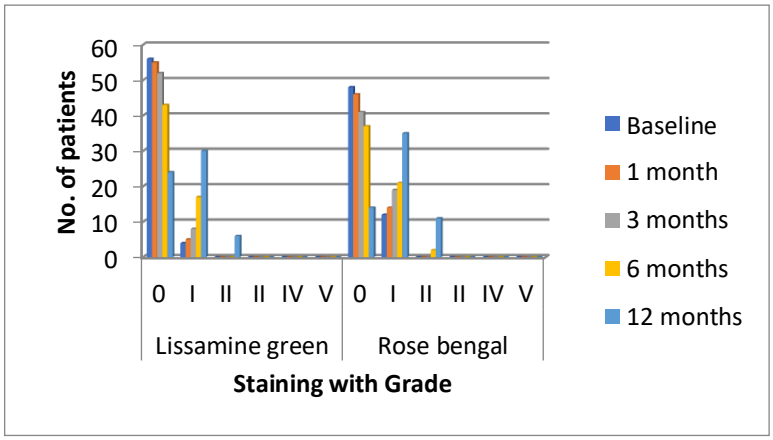

Figure 28: Graphical representation of changes in conjunctival and corneal staining in group I on follow up

Number of eyes with ocular surface staining as well as grades of both lissamine green and rose bengal staining increased on follow up visits as compared to baseline grades (Table 11),(Figure 28)

Table 12: Changes in Conjunctival impression cytology on follow up in group I

Conjunctival impression cytology		Follow up				
		Baseline	1 month	3 months	6 months	12 months
		n (%)	n (%)	n (%)	n (%)	n (%)
Goblet cell density (cells/hpf)	>75	56 (93.34%)	56 (93.34%)	48 (80%)	20 (33.33%)	20 (33.33%)
	50-75	4 (6.66%)	4 (6.66%)	12 (20%)	30 (50%)	30 (50%)
	15-50	0	0	0	10 (16.66%)	10 (16.66%)
	<15	0	0	0	0	0
Squamous metaplasia	Mild	4 (6.66%)	4 (6.66%)	16 (26.66%)	40 (66.66%)	40 (66.66%)
	Moderate	0	0	2 (3.33%)	15 (25%)	15 (25%)
	Severe	0	0	0	0	0
	No change	56 (93.34%)	56 (93.34%)	42 (70%)	5 (8.34%)	5 (8.34%)
Inflammatory cells	Present	0	0	0	36 (60%)	36 (60%)
	Absent	60 (100%)	60 (100%)	60 (100%)	24 (40%)	24 (40%)

Number of eyes with goblet cell density ≤ 75 cells/hpf reduced on subsequent follow up visits compared to baseline cell count. At the beginning of the study 4 eyes had goblet cell density 50-75 cells/hpf, which increased to 12 eyes at 3 month and 30 eyes at 6 months and 12 months follow up. None of the eyes had goblet cell density 15-50 cells/hpf at the beginning of the study but at 6 months follow up 10 eyes had goblet cell density 15-50 cells/hpf. During the course of 1 year follow up none of the eyes had goblet cell density <15

cells/hpf. Similarly at baseline, 56 eyes showed no squamous metaplasia while 4 had mild degree of squamous metaplasia. At 6 months and 12 months follow up only 5 eyes showed no squamous metaplasia while number of eyes in both mild and moderate degree increased with 40 eyes showing mild and 15 eyes showing moderate degree of squamous metaplasia. None of the eyes showed the presence of inflammatory cells till 3 months of follow up. At 6 months follow up 36 eyes showed inflammatory cells (Table 12).

Table 13: Changes in Ocular surface parameters in group II on follow up

Parameters	Baseline	1 month	3 months	6 months	12 months	p value
OSDI Questionnaire score	35.17±5.98	37.79±6.11	40.64±5.53	44.37±5.32	48.56±5.81	<0.0001
Schirmer's test	13.91±3.03	12.98±2.90	11.68±2.46	10.21±2.49	8.81±2.15	<0.0001
TBUT	8.5±1.72	7.31±1.65	6.43±1.38	5.5±1.11	4.46±1.08	<0.0001

In group II, mean values Schirmer's test and TBUT significantly decreased ($p < 0.0001$) and the mean value of OSDI score significantly increased on follow up when compared to baseline mean values (Table 13).

Table 14: Changes in corneal sensitivity in group II on follow up

Corneal sensitivity	Follow up				
	Baseline	1 month	3 months	6 months	12 months
	n (%)	n (%)	n (%)	n (%)	n (%)
Intact all quadrant	59 (98.33%)	59 (98.33%)	58 (96.67%)	58 (96.67%)	58 (96.67%)
Reduced	1 (1.67%)	1 (1.67%)	2 (3.33%)	2 (3.33%)	2 (3.33%)

No significant difference was seen in corneal sensitivity on follow as compared to baseline (Table 14).

Table 15: Changes in tear meniscus parameters on AS-OCT on follow up in group II

Tear meniscus parameters	Baseline	1 month	3 months	6 months	12 months	p value
TMH	248.93±6.95	246.65±7.18	243.35±7.27	239.71±7.68	236.13±7.45	<0.0001
TMD	149.9±6.30	147.68±6.35	144.31±6.26	140.93±6.13	137.46±6.55	<0.0001
TMA	0.018±0.001	0.018±0.001	0.017±0.001	0.016±0.001	0.016±0.001	<0.0001

(AS-OCT- Anterior segment-Optical coherence tomography; TMH-Tear meniscus height; TMD-Tear meniscus depth; TMA-Tear meniscus area)

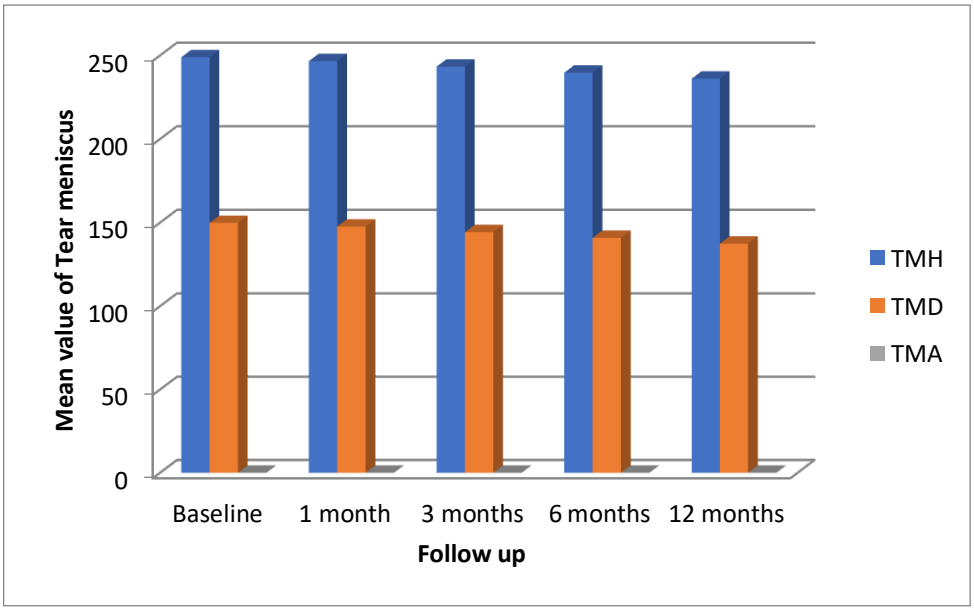

Figure 29: Graphical representation of changes in tear meniscus parameters on follow up in group II

The mean values of tear meniscus height, tear meniscus depth and tear meniscus area significantly decreased on follow up visits as compared to baseline values (Table 15),(Figure 29). Mean value of TMH in eyes on timolol at 1 year follow up was 230.16±11.54 and for eyes on prostaglandin analogue at 1 year follow up was 232.344±6.49,(p= 0.6947).

Table 16: Changes in conjunctival and corneal staining in group II on follow up

Staining	Grade	Follow up				
		Baseline	1 month	3 months	6 months	12 months
		n (%)	n (%)	n (%)	n (%)	n (%)
Lissamine green	0	21 (35%)	14 (23.33%)	5 (8.33%)	0	0
	I	35 (58.88%)	41 (68.33%)	36 (60%)	13 (21.67%)	0
	II	4 (6.67%)	5 (8.33%)	17 (28.33%)	35 (58.33%)	30 (50%)
	II	0	0	2 (3.33%)	12 (20%)	27 (45%)
	IV	0	0	0	0	7 (11.67%)
	V	0	0	0	0	0
Rose bengal	0	13 (21.67%)	3 (5%)	1 (1.67%)	2 (3.33%)	0
	I	37 (61.67%)	41 (68.33%)	28 (46.67%)	35 (58.33%)	0
	II	10 (16.67%)	16 (26.67%)	26 (43.333%)	23 (38.33%)	14 (23.33%)
	II	0	0	5	0	35 (58.33%)
	IV	0	0	0	0	11 (18.33%)
	V	0	0	0	0	0

Number of eyes with increasing grades of both lissamine green and rose bengal staining increased on follow up visits as compared to baseline grades, (Table 16).Eyes on timolol showed higher grade of lissamine staining at 1 year follow up as compared to prostaglandin analogues (max grade 3).

Table 17: Changes in Conjunctival impression cytology on follow up in group II

Conjunctival impression cytology		Follow up				
		Baseline	1 month	3 months	6 months	12 months
		n (%)	n (%)	n (%)	n (%)	n (%)
Goblet cell density (cells/hpf)	>75	3 (5 %)	3 (5%)	2 (3.33%)	2 (3.33%)	1 (1.66%)
	50-75	24 (40%)	22 (36.66%)	23 (38.34%)	22 (36.67%)	20 (33.33%)
	15-50	14 (23.33%)	16 (26.67%)	15 (25%)	16 (26.67%)	17 (28.34%)
	<15	19 (31.67%)	19 (31.67%)	20 (33.34%)	20 (33.33%)	22 (36.67%)
Squamous metaplasia	Mild	21 (35%)	20 (33.33%)	18 (30%)	15 (25%)	15 (25%)
	Moderate	23 (38.33%)	23 (38.33%)	23 (38.33%)	25 (41.67%)	20 (33.33%)
	Severe	16 (26.67%)	17 (28.34%)	19 (31.66%)	20 (33.34%)	25 (41.67%)
Inflammatory cells	Present	53 (88.33%)	53 (88.33%)	56 (93.33%)	57 (95%)	59 (98.33%)
	Absent	7 (11.67%)	7 (11.67%)	4 (6.67%)	3 (5%)	1 (1.67%)

Impression cytology specimens showed reduction in goblet cell density , increment in severity of squamous metaplasia and appearance of inflammatory cells on follow up as compared to baseline (Table 17).

DISCUSSION

DISCUSSION

Dry eye is one of the major complications of anti-glaucoma medications which has a negative impact on management of glaucoma patients. Many studies previously had established the role of topical anti-glaucoma medication (AMG) in effective management of primary open angle glaucoma. Anti-glaucoma medical treatment is a lifelong therapy; hence it exposes the ocular surface to the harmful effects of topical IOP lowering medications. In our study we enrolled 120 eyes of 60 POAG patients, out of which 60 eyes were treatment naïve while rest 60 were on topical AGM. We assessed the difference in various ocular surface parameters between the two study groups.

In our study, the value of Schirmer test was significantly lower in group 2 as compared to group 1 at baseline ($p<0.001$). Few other studies also found similar results.[68],[69] At 1 year follow up, the Schirmer's test values deteriorated significantly in both the groups ($p< 0.0001$). This was in concordance with the study done by Kamath et al.[6] In group II, eyes on ≥3 topical AMG per day at baseline had significantly lower Schirmer's test values ($p <0.0001$) as compared to eyes on < 3 AMG per day. This could be due to increased exposure to higher concentrations of anti-glaucoma medications and their preservatives. This was in contrast to study done by Ramli et al[7] in which no increased difference in Schirmer test value was see as the number of eye drops increased. In group II, Schirmer test values did not show any significant difference depending upon the duration of treatment. Pai et al [59], found similar results as our study along with lower Schirmer test values in eyes with the duration of treatment ≥ 5 years.

We found significantly lower TBUT values in group II as compared to group I at baseline ($p <0.0001$). Similar to Schirmer test values, the TBUT values also reduced significantly ($p <0.0001$) at follow up visits in both the groups. TBUT values at baseline were poorer in eyes on ≥3 AMG per day and with duration of treatment ≥3 years. This was in correspondence with the studies few studies done earlier.[70] In the study done by Pérez-Bartolomé F et al [54], no significant difference was noted in NI-TBUT ($p>0.05$) between the anti-glaucoma medication group and controls.

The difference in OSDI score was not statistically significant between the two groups in our study. Similarly, Went et al[64] and Cvenkel et al[70] also found no significant difference in OSDI score between treated and untreated glaucoma patients. This could be due to the fact

that in our study region the factors like high temperature and windy climate were more prevalent, which can lead to higher OSDI scores in both the groups. But, the OSDI score significantly increased at 1 year follow up in both the groups. Study done by *Pérez* et al[54], found lower OSDI score with the use of preservative free eyedrops. Studies done by *Ramli et al*[7] and Lajmi et al[55] found that BAK was the major factor associated with higher OSDI score. Ramli *et al* [7] found a significantly higher OSDI score in glaucoma group (p = 0.028) as compared to controls along with rates of abnormal OSDI nearly three times more in subjects who were on BAK containing antiglaucoma drops but they found no correlation in increased differences in OSDI results and the number of eye drops.

Difference in corneal sensations at baseline between the groups did not show any statistically significant difference (p=1.00). Also, on 1 year follow up, corneal sensations showed no significant reduction in both the groups. This was in contrast to the study done by Went et al [64] who found lower corneal sensitivity in patients on preserved anti-glaucoma medications as compared to untreated patients (P < 0.001). Possible explanation for this could be the qualitative nature of the method we used to assess the corneal sensitivity. Saini M et al[61], assessed central corneal sensations and central subbasal nerve fiber layer density. In this study, the central corneal sensations were decreased in eyes on antiglaucoma drops as compared to the controls (P=0.076).Along with this, the FTBUT (R2=0.84), corneal sensitivity (R2=0.52) showed positive correlation to central corneal SBNFLD in the long term topical antiglaucoma medication group.

In our study we found a significantly higher (p < 0.0001) mean values of TMH and TMD in group II as compared to group I at baseline. At 1 year follow up , in group I, the mean values of THM and TMD reduced significantly (p<0.05) but the decrease in mean value of TMA was statistically insignificant (p>0.05) as compared to baseline values. In group II, at I year follow up the mean values of TMH,TMD and TMA reduced significantly (p=0.0001) when compared to baseline. Wong ABC et al [62] measured tear meniscus height by taking three measurements near the centre of the lower meniscus with pre-calibrated digital imaging and then averaging them, and found that TMH was significantly poorer in treated eyes as compared to fellow eye (p=0.03) but the long term effects on tear meniscus were not assessed by them since that was a cross-sectionl study.

Study done by Agnifili et al[50] compared tear meniscus parameters in medical controlled glaucoma patients, patients with evaporative dry eye and healthy controls and found similar

results as our study. They also assessed upper tear meniscus parameter ,which was not done in our study.Pérez et al[54] found no significant difference in L-TMH between the treatment group and controls (p>0.05) but L-TMH (R -0.142; p = 0.043) was found in higher daily preservative concentration of topical AGM.

Goblet cell density was significantly low in group II as compared to group I at baseline (<0.0001). None of the eyes in group I had inflammatory cells while 53 eyes (88.33%) had inflammatory cells in group II at baseline (<0.0001).Amount and severity of squamous metaplasia was significantly higher (<0.0001) in group II as compared to group I at baseline. At 1 year follow up, as compared to baseline goblet cell density reduced, amount and severity of squamous metaplasia increased in both the groups. As compared to baseline, the number of eyes showing the presence of inflammatory cells increased in both the groups. Yalvaç IS et al[68] found a significant degree (p <0.001) of squamous metaplasia (graded according to Nelson grading) and a significant (p <0.001) reduction in goblet cell density in the treatment group as compared to controls. A study done by Brandt JD et al[37] was different since they took glaucoma suspects who were not on topical anti-glaucoma medications as controls. They found a statistically significant increase in cytologic grade in the treatment group compared to the controls and a statistically significant degree of conjunctival metaplasia was associated with the number of glaucoma medications used.

Hong et al, Arici et al [69] and Cvenkel et al[70] graded the impression cytology specimen according to Nelson grading and found significantly higher scores in the medication group as compared to the control group.

Grades of ocular surface staining by both lissamine green and rose bengal stains were significantly (p<0.0001) lower in group I as compared to group II. At 1 year follow up, grades of both lissamine green and rose bengal ocular surface staining increased in both the groups.Study done by Pérez et al[54], Ramli et al[7] also found similar results as our study but they used fluorescein dye to stain the ocular surface. In our study we used lissamine green and rose bengal stains because these dyes are poorly seen within the tear film; hence does not obscure the staining pattern. Also, because they do not diffuse into the substantia propria of the conjunctiva, the staining pattern is retained for longer duration at high contrast . Lajmi et al[55] used Fluorescein and a lissamin green dyes found that statistically lower incidence of superficial punctate keratitis (SPK) and corneal and conjunctival staining in the lissamine green test (P < 0.001) was associated with fixed combination of anti-glaucoma eyedrop.

CONCLUSION & LIMITATIONS

CONCLUSION

1. A total of 120 eyes of 60 POAG patients were examined of which 60 eyes were treatment naïve while other 60 eyes were already on topical AGM.
2. Mean age was 59.00 ±11.52 years in group I and 60.3±9.31years in group II.
3. Mean visual acuity, baseline IOP, CDR and mean deviation on HVF were 0.344±0.618, 19.66 ± 7.21mmHg, 0.71±0.164, 5.899±2.69 respectively in group I and 0.59 ± 0.998, 17.13 ± 6.209mmHg, 0.733±0.176, 6.64 ± 2.41 respectively in group II.
4. Mean values of Schirmer's test, TBUT, OSDI score were 20.2±3.02mm, 12.1±2.31seconds, 34.25±9.02 respectively in group I and 13.91±3.03mm, 8.5±1.72 seconds, 35.17±5.98 respectively in group II at baseline.
5. Only one eye in group II had reduced corneal sensations while corneal sensations were intact in all the eyes in group I at baseline.
6. Both lissamine green and rose Bengal staining showed higher grades in group II compared to group I at baseline.
7. Mean values of TMH, TMD, TMA were 253.33±8.86 µ, 154.05±5.85 µ, 0.025±0.029mm^2 respectively in group I while in group II 248.93±6.95µ, 149.9±6.30 µ, 0.018±0.001 mm^2 respectively in group II at baseline.
8. Goblet cells density was lower with higher grade of squamous metaplasia along with precence of inflammatory cells in group II as compared to group I at baseline.
9. At 1 year follow up the mean values of schirmer's test, TBUT, OSDI score decreased significantly in both the groups.
10. At 1 year follow up, corneal sensitivity was intact in all the eyes while only 2 eyes had reduced corneal sensations in group II.
11. At 1 year follow up, mean values of TMH, TMD, TMA reduced significantly in both the groups.
12. At 1 year follow up, grades of both lissamine green and rose bengal ocular staining increased in both the groups.
13. At 1 year follow up, reduction in goblet cells density, increament in degree of squamous metaplasia along with presence of inflammatory cells were seen in both groups.

Hence, it is very essential to diagnose dry eye at an early stage for an effective management. The tests employed for diagnosis of dry eye should be non-invasive to minimally invasive, needs lesser time to perform and has good reliability and reproducibility.AS-OCT in this

aspect is a reliable and noninvasive tool to image the tear meniscus in chronically glaucomatous patients.

LIMITATIONS

1. Factors like high temperature and windy climate were more prevalent in our study region. So this could be one of the confounding factors.
2. We could not assess the effect of individual drugs on ocular surface since most of the patients in our study were on >1 topical AGM.

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BIBLIOGRAPHY

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ANNEXURES

ANNEXURE I

अखिल भारतीय आयुर्विज्ञान संस्थान, जोधपुर
All India Institute of Medical Sciences, Jodhpur
संस्थागत नैतिकता समिति
Institutional Ethics Committee

No. AIIMS/IEC/2021/ 357 6 **Date: 12/03/2021**

ETHICAL CLEARANCE CERTIFICATE

Certificate Reference Number: **AIIMS/IEC/2021/3351**

Project title: **"Effect of topical anti-glaucoma medications on ocular surface: a prospective cohort study"**

Nature of Project:	**Research Project Submitted for Expedited Review**
Submitted as:	**M.D. Dissertation**
Student Name:	**Dr. Krati Srivastava**
Guide:	**Dr. Kavita R Bhatnagar**
Co-Guide:	**Dr. Pankaja Ravi Raghav, Dr. Poonam Elhence & Dr. Jyoti Shakrawal Varshney**

Institutional Ethics Committee after thorough consideration accorded its approval on above project.

The investigator may therefore commence the research from the date of this certificate, using the reference number indicated above.

Please note that the AIIMS IEC must be informed immediately of:

- Any material change in the conditions or undertakings mentioned in the document.
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research.

The Principal Investigator must report to the AIIMS IEC in the prescribed format, where applicable, bi-annually, and at the end of the project, in respect of ethical compliance.

AIIMS IEC retains the right to withdraw or amend this if:

- Any unethical principle or practices are revealed or suspected
- Relevant information has been withheld or misrepresented

AIIMS IEC shall have an access to any information or data at any time during the course or after completion of the project.

Please Note that this approval will be rectified whenever it is possible to hold a meeting in person of the Institutional Ethics Committee. It is possible that the PI may be asked to give more clarifications or the Institutional Ethics Committee may withhold the project. The Institutional Ethics Committee is adopting this procedure due to COVID-19 (Corona Virus) situation.

If the Institutional Ethics Committee does not get back to you, this means your project has been cleared by the IEC.

On behalf of Ethics Committee, I wish you success in your research.

Dr. Praveen Sharma
Member Secretary

Member secretary
Institutional Ethics Committee
AIIMS,Jodhpur

Basni Phase-2, Jodhpur, Rajasthan-342005; **Website:** www.aiimsjodhpur.edu.in; **Phone:** 0291-2740741 Extn. 3109
E-mail : ethicscommittee@aiimsjodhpur.edu.in; ethicscommitteeaiimsjdh@gmail.com

ANNEXURE II

Proforma

Group 1/Group 2 **Date-**

Name: **Age:**

Registration Number: **Contact number:**

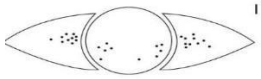
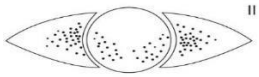
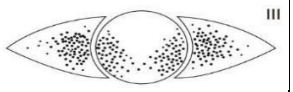
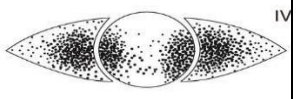
Address:

	Day-1	**1 month**	**3 months**	**6 months**	**12 months**
Presenting complaints					
Past complaints					
Personal history					
Family history					
OSDI Questionnaire score (Appendix-1)					
Visual aquity (Unaided)- Distance	RE: LE:				

Visual aquity (Unaided)-Near	RE: LE:				
Visual Aquity(Best corrected)	RE: LE:				
Refraction-	RE: LE:				
IOP (mm Hg) -	RE: LE:				
CCT (µm)-	RE: LE:				
Anterior segment-	RE: LE:				
Fundus-	RE: LE:				
<u>Ocular Surface Findings:</u>					
Schirmer's test (Type1)-	RE: LE:				
Tear Film Break Up Time Test-	RE: LE:				

Corneal sensitivity-	RE: LE:				
Conjunctival hyperemia	RE: LE:				
AS-OCT Parameters:					
Tear meniscus height	RE: LE:				
Tear meniscus depth	RE: LE:				
Tear meniscus area.	RE: LE:				
Conjunctival Impression Cytology Findings:					
Goblet cells density	RE: LE:				
Squamous metaplasia	RE: LE:				

Presence or absence of inflammatory cells.	RE: LE:				
Conjunctival and corneal Staining -	RE: LE:				

Panel	Staining Pattern	Grade	Criteria
A		0	Equal to or less than panel A
B		I	Equal to or less than panel B, greater than panel A
C		II	Equal to or less than panel C, greater than panel B
D		III	Equal to or less than panel D,greater than panel C
E		IV	Equal to or less than panel E, greater than panel D
>E		V	Greater than panel E

Ocular Surface Disease Index (OSDI)

Circle the number in the box that best represents each answer. Then fill inboxes 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. Eyes that feel gritty?	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

Subtotal score for answers from 1-5: ( )

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	
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?	4	3	2	1	0	N/A
9. Watching TV?	4	3	2	1	0	N/A

Subtotal score for answers from 6-9:( )

Have your eyes felt uncomfortable 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	
10. Windy conditions?	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

Subtotal score for answers from 10-12:( )

Add subtotal A, B, C to obtain D-

(D=Sum of scores for all questions answered)

Total number of questions answered (Do not include questions not answered)

$$\text{OSDI} = \frac{\text{SUM OF SCORES FOR ALL THE QUESTIONS ASKED}}{\text{TOTAL NUMBER OF QUESTIONS ANSWERED}} \times 25$$

ANNEXURE III

All India Institute of Medical Sciences

Jodhpur, Rajasthan

Informed Consent Form

Title of Thesis/Dissertation: **Effect of topical anti-glaucoma medications on ocular surface: a prospective cohort study**

Name of PG Student: Dr. Krati Srivastava Tel. No.7597126994/_____

Patient/Volunteer Identification No._____ I, _____D/o_____ R/o

give my full, free, voluntary consent to be a part of the study **"Effect of topical anti-glaucoma medications on ocular surface: a prospective cohort study"**, the procedure and nature of which has been explained to me in my own language to my full satisfaction. I confirm that I have had the opportunity to ask questions. I understand that my participation is voluntary and am aware of my right to opt out of the study at any time without giving any reason. I understand that the information collected about me and any of my medical records may be looked at by responsible individual from Department of Ophthalmology, ALL INDIA INSTITUTE OF MEDICAL SCIENCES (AIIMS) or from regulatory authorities. I give permission for these individuals to have access to my records.

Signature/Left thumb impression

This to certify that the above consent has been obtained in my presence.

Date:_____Place: _____

Signature of PG Student

Witness 1	Witness 2
Signature	Signature
Name:_____	Name:_____
Address:_____	Address:_____
Phone no_____	Phone no_____

ANNEXURE IV

अ िर बायतीम ि ित्सा

ि ान सोस्थान

जोधनुय, याजस्थान

सू ित सहभतत प्रत्र

थी सस/तनफोध ि ा शीर्ष : साभतमि ऐटी-ग्र ू ोभा दिाओ

ि ा ऑक्मू य सयपे साय प्रबाि: एि प्रोस्नोक्टि ि ोहोटष

नीजी छात्रा ि ा नाभ: डॉ. ि ृ तत श्रीिास्तव

दयबाय्। सोख्मा 7597126994 /

योगी / स्मिसेिी ि ी नहिान._____

भ,ैं _____नुत्री_____अध्ममन ि ा एि हहस्सा फनने ि े रए भेयी

न , स्तोत्र, स्ैक्छछि सहभतत व्मक्त ि यता/ि यती हू। थी सस / "साभतमि ऐटी-तनफोध ि ा शीर्ि ग्र ि ोभा दिाओ ि ा ऑक्मूरय सयपे स नय प्रबाि: एि प्रोस्नोक्टि ि ोहोटष अध्ममन।" क्जस ि ी प्र ि मा औय प्र ि ृ तत ि ो ी सोतुक्टट ि े रए भेयी अनुनी बाय्ा भें भुझे अनुनी न नुक्टट सभझामा गमा है। भने ि ा अिसय भया है। ि यता/ि यती हू ो ि भुझे प्रश्न नछू

भैं सभझत ा/ सभझती हू ो ि भेयी बागीदायी स्ैक्छछ ि है औय भुझे ि ोई बी ि ायर् हदए बफना ि सी बी सभम अध्ममन से फाहय तन ि रने ि े भेये अ धि ाय ि ी जानि ायी है।

भैं सभझत ा/ सभझती हू ो ि भेये औय भेये भे डिर य ि ॉडष ि े फाये भें एि ित्त ि ी गई जानि ायी ि ो नेत्र ि ान ि बाग, अ िर बायतीम ि ि त्सा ि ान सोस्थान से क्जम्भेदाय व्क्त दृ ि ाया दे ि ा जस ि ता है। भैं इन व्क्तमों ि ो अनने अ बरे ि ों ति नहु ो ि ि े रए अनु भतत दे ता /दे ती हू हू ो।

हस्ता ंय / फाऐ अोगठे ि ा छात्र

मह प्रभा रत ि यने ि े रए ि भेयी उनक्स्थतत भें उनयोक्त सहभतत प्राप्त ि ी गई है। ताय ीि : _____स्थान: __

नीजी छात्र िे हस्ताांय

1. **स**ाांी 1

हस्ताांय :

नाभ:_____

नता :_____

फोन नॊफय:_____

2. **स**ाांी 2

हस्ताांय:

नाभ: _____

नता : _____

फोन नॊफय:_____

ANNEXURE V

PATIENT INFORMATION SHEET

Title of Thesis/Dissertation: Effect of topical anti-glaucoma medications on ocular surface: a prospective cohort study

You are invited to take part in this research study. Before you decide whether to take part it is important for you to understand why the research is being done and what will it involve. Please take your time to read the information and then decide. Queries if any will be addressed. This study aims to compare the difference between ocular surface changes, anterior segment optical coherence tomography (AS-OCT) and conjunctival impression cytology and ocular staining findings in newly diagnosed cases of primary open angle glaucoma and patients of primary open angle glaucoma on topical anti-glaucoma medications.

1) Why have I been chosen to take part in the study?

You have been chosen to take part in the study because you are a diagnosed case of Primary Open Angle Glaucoma not on Topical Anti-glaucoma medications or diagnosed cases of Primary Open Angle Glaucoma on Topical Anti-glaucoma medications. You will be evaluated for different parameters as specified in the title of the study apart from a basic ophthalmological examination.

2) What is the purpose of the study?

The purpose of the study is to carry out ophthalmological examination in newly dignosed cases of Primary Open Angle Glaucoma (not on any topical anti-glaucoma medications) and patients of primary open angle glaucoma on Topical Anti-glaucoma medications. You will have to fill OSDI questionnaire and basic ophthalmological examination including the Visual acuity, Refraction, IOP, CCT, Anterior Segment under Slit-lamp bi-microscopy, Fundus, Schirmer's Test type-I, Tear film break up time test, corneal sensitivity, AS-OCT, Conjunctival impression cytology, ocular surface staining will be done. The main aim is to find out a significant difference, if any.

3) Do I have to take part in the study?

It is up-to you to decide whether or not to take part in the study. In case you decide to take part, you will be given the information sheet and will be asked to sign the consent form.

If you decide to take part, you can still withdraw your consent anytime in the study without giving any reasons.

4) What will happen to me if I take part in the research?

This study is a prospective cohort study, which means you have to visit hospital at 1month, 3months, 6months and 12 months interval. You will be subjected to history taking and examination. You will have to fill OSDI questionnaire and the examination will involve measuring your Visual acuity, Refraction, IOP, CCT, Anterior Segment under Slit-lamp bimicroscopy, Fundus, Schirmer's Test type-I, Tear film break up time test, corneal sensitivity, conjunctival hyperaemia .You will then be examined under OCT machine to look for various anterior segment parameters like Tear meniscus height, Tear meniscus depth, Tear meniscus area. In addition to this, Conjunctival impression cytology, ocular surface staining will also be done. All these procedures are non-invasive.

5) What do I have to do?

You will have to sign an informed consent form and read the patient information sheet provided. You will have to cooperate for Visual acuity, Refraction IOP, CCT, Schirmer's Test, Tear Break Up Time, OSDI questionnaire, corneal sensitivity, AS-OCT, Conjunctival impression cytology, ocular surface staining. This is a prospective cohort study, so follow up at 1 month,3 months, 6 months and 12 months interval will be required from your side.

6) What are the possible benefits of taking part in the study?

Topical anti-glaucoma medications can lead to various changes in ocular surface, which can induce various discomforting symptoms, which eventually can lead to decrease compliance to the treatment. Your various ocular surface parameters will be evaluated and you will be screened for abnormalities in all those parameters.

7) What are the possible side effects of taking part in the study?

There are no additional side effects of taking part in the study.

8) Will my data be kept confidential?

Your medical records and demographic data will be disclosed only to the researcher, treating physician and concerned authorities

ANNEXURE VI

योगी सूचना पत्रक

थी सस / तनफोध : साभतमि ोभा दिाओं ि्ा य सयपे स
ि्ा शीर्ि एोटी-ग्रस्ि ऑक्म

नय प्रबाि: एि प्रोस्ोक्टि ि्ोहोटष अध्ममन।

आन्ि्ो इस शोध अध्ममन भेंं बाग रेने ि्ै रए आभोक्त ि्मा जाता है।बाग रेना है मा नही, मह तम

ि्यने से नहरे मह आन्ि्ै रए सभझना भहत्ि्न ष है ि्ि शोध क्मों ि्मा जा यहा है औय इसभेंं क्मा

शा भर होगा। ि्ृ नमा जानि्ायी नढ़ने औय पय तम ि्यने ि्ै रए अन्ना सभम रेंं। ि्सी बी सिार

ि्ो सोफो धत ि्मा जाएगा। इस अध्ममन ि्ा उद्देश्म ,न्न ार
ओि्ु रय सतह भेंं नरयितन ि् ि्ोड

ऑप्टिर ि्ॉइयेंस टोभोग्राफी (एएस-ओसीटी),नेत्श्रेटभरा ि्ो शि्ा छान्न, ओि्ु रय सतह छान्न ि्ै

तनटि्र्ों ि्ी तुरना प्राथ भि ोभा ि्ै नए भाभरे औय ोभा
ओन्नन ऐगर ग्रस्ि सााभतमि ऐटी-ग्रस्ि

दिाओं नय प्राथ भि ओन्नन ऐगर ग्रस्ि ोभा ि्ै यो गमों ि्ी जाएगी ।

1) अध्ममन भेंं बाग रेने ि्ै रए भुझे क्मों ि्ुना गमा है?

आन्ि्ो अध्ममन भेंं बाग रेने ि्ै रए ि्ुना गमा है क्मों ि्ि आन्न मा तो प्राथ भि ओन्नन ऐगर

ग्र ोभा ि्ै नए भाभरे हैंं मा प्राथ ोभा ि्े एि ांात भाभरे हैं।
ि् भस्ि ओन्नन ऐगर ग्रस्ि आन्नो एि

फुन्नमादी नेत्र ि्ान्न नयींंा ि्ै अराि्ा अध्ममन ि्ै शीर्ि भेंं तनहदटष्ठ ि् बन्न भान्नदडों ों ि्ै रए

भलमाों ि्न ि्मा जाएगा।

2) अध्ममन िंा उद्देश्म क्मा है?

अध्ममन िंा उद्देश्म प्राथ भि ओन्नन ऐगर ोभा यो गमों भें एि फुतनमादी नेत्र ग्रहि न्नयीं्ा िंयना है।

ओिंु रय सतह भें न्नरयितन न्नता िंयने िंि रए शभय टे स्ट, आोसू पलभ ब्रेि अन्न टाइभ टे स्ट,

िंॉतनमर सोिंेदनशीरता िंा न्नयीं्र् िंमा जाएगा तथा ऑसडी प्रश्नाि्रीबये जाएोगे।इसिंै अराि्ंा

न्न िंार ि्ोड ऑप्टिर िंॉइयें स टोभोग्रापी (एएस-ओसीटी),नेत्रश्रेटभरा िंो शि्ंा ि्ी छान्न, ओिंु रय

सतह ि ी छ ान र ज ाएग ी।इस ि ै अर ा ि ा प ो डस न यी ं ा होग ा, आईओन ी रमा ज ामे ग ा। भ ु ख् म उद्देश्म

इन यो गम ो ं भे ं दृश्म हातन ि े रए ष अ ो तय, महद ि ो ई ह ो, औय तनिायि य र्न ीततम ो ं ि ा
ए ि भहत् ि न न ता रगाना है।

3) क्मा भुझे अध्ममन भें हहस्सा रेना जरूयी है?

मह तम ि यना ह ै ि आन ा ि ो ब ाग र े ना ह ै मा नहीं। महद आन रेते हैं तो आन ो ब ाग र े न े ि ा तनर् म

स ि न ा न त्र हदमा ज ाएग ा औय सहभतत न त्र न य हस्त ा ं य ि यन े ि े रए ि ह ा जाएग ा। महद आन ब ाग

र े न े र े ते ह ै ं तो आन बफना ि सी ि ायर् ि े अध्ममन भे ं ि बी ब ी अन न ी सहभतत ि ान स रे
ि ा

तनर् स ि ते

हैं।

4) महद भैं अध्ममन भें बाग रेता हॊ तो भेये साथ क्मा होगा?

मह अध्ममन ए ि प्र ो स्न े क्क ट ि ो ह ो ट ष अध्ममन ह ै क्जस ि ा अथष ह ै ि

आन ा ि ो 1 भहीन े , 3 भहीन े ,6भहीन े औय 12 भहीन े ि े अ ो तयाय न य अस्न त ा र आना

ह ै। न य ी ं ा भे ं आन ि ै ओ ि ु र य सतह भे ं न रयितष न ता ि यन े ि े रए

शभय ट े स्ट, आ ो स ू पलभ ब् े ि अन टाइभ ट े स्ट, ि ॉतनमर स ो ि े दनशीरत ा

ि ा

न य ी ं र् ि मा ज ाएग ा तथा ऑसडी प्रश्नाि ी बय े ज ाए ो ग े ।इस ि ै अर ा ि ा न ि ू ि ार ि ो ड ऑप्टिर

ि ॉइय े ं स टोभ ो ग्र ा प ी (एएस-ओस ी ट ी),न े त्रश्र े टभरा ि ो श ि ा ि ी छ ान ,

ओ ि ु र य सतह ि ी छ ान र ज ाएग ी।इस ि ै अर ा ि ा प ो डस न य ी ं ा होग ा,

आईओन ी रमा ज ामे ग ा। म े सब ी प्र ि माए ो ग ै य-आ ा भि ह ै ं।

5) भुझे क्मा ि◌ियना होगा?

आन्नो आईओन्नी, सीसीटी औय शभय ि◌ेे टेस्ट औय ऑसू पलभ ि◌ी श थरता, न्नोस्टीरयमय सेगभेंट इभेज्जोग ि◌ैे रए सहमोग ि◌ियना होगा। आन्नि◌ो एि सू ि◌ित सहभतत पॉभष न्नय हस्ताक्षय ि◌ियना होगा

औय आन्नि◌ो प्रदान ि◌ी गई योगी सि◌ना न्नत्र ि◌ो न्नढ़ना होगा।

6) अध्ममन भें बाग रेने ि ेसोबा ित राब क्मा हैं?

प्ाथ भि ओन्नन ऐगर ोभा तथा साभतमि ोभा दिाइमाॉ आॉि ि ै
ग्रि ऐटी-ग्रि ओि ु रय सतह भेंं

न्नरयितन, ार ि ॉड ,नेत्रश्रेटभरा ि ो शि ा तथा ओि ु रय ि ा
न्नू सतह छान्न भेंं न्नरयितन ि ायर् फन
ि

सि ता है, क्जस न्नय ि ई फ ाय ि सी ि ा ध्मान नहीं जाता है। आन्नि ै
ओि ु रय सतह , न ि ार

ि ॉड,नेत्रश्रेटभरा ि ो शिा, ओि ु रय सतह छान्न ि ा भलमाॉि न ि मा
जाएगा औय आन्नि ो उन सबी

भान्नदॉडों भेंं असाभान्मताओं ि ै रए स्ि ीन ि मा जाएगा।

इस ि ै अराि ा, महद अध्ममन ि ै फाद ि ोई भहत्ि न्नूर्ष
तनटि र्ष भरता है, तो आन्नि ो तनायि ियर्नीततमों ि ै अधीन
ि मा जाएगा औय म्हआिश्मि हो, तो आन्न प्ायॉ बि उन्नाय ि ै रए ए
उम्भीदि ाय होंगे।

7) अध्ममन भेंं बाग रेने ि ै सॉबा ित दटु प्रबाि क्मा हैंं?

अध्ममन भेंं बाग रेने ि ै ि ोई बी अततरयक्त दटु प्रबाि नहीं हैंं।

8) क्मा भेया डेटा गोन्ननीम यिा जाएगा?

आन्नि ै भे ड ि र रयिॉडष औय जनसॉख्मिीम डेटा ि ा ि ै ि र
शोधिाष, ि ि त्सि औय सॉफो ध्ाअ धि ारयमों ि ै साभने
ि ुरासा ि मा जाएगा।