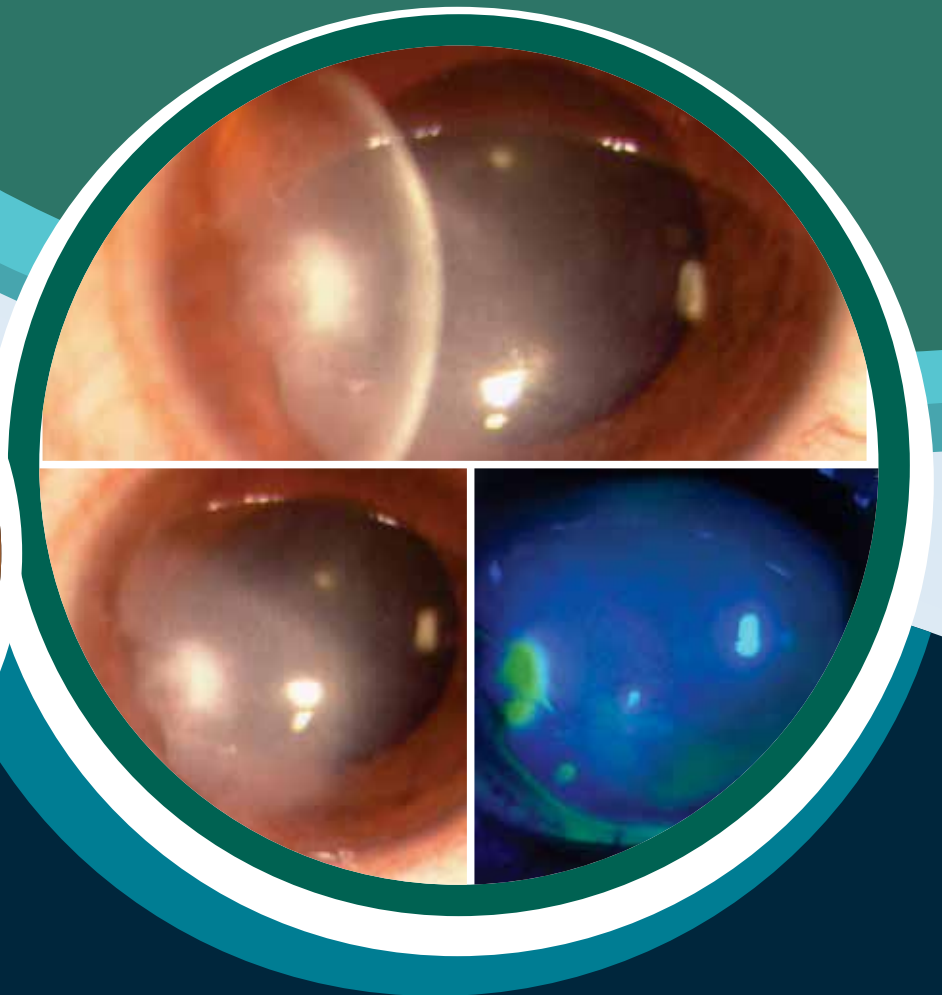


# KeraSight

Official Publication of Indian Society of Cornea  
and Keratorefractive Surgeons

Volume 3; September - December 2017



## Special Features

*Vital Dyes For Keratorefractive Surgeons*

*Nocardia Scleritis*

*Post Surface Ablation Haze*

*Types of Riboflavin & Methods of Corneal Crosslinking*



INDIAN SOCIETY OF CORNEA  
AND KERATOREFRACTIVE SURGEONS

# Moxicip

Moxifloxacin 0.5%

**Livewire for Infection Control**

**A global & trusted quality Brand Offering 24x7 hour protection**

- The most Widely Prescribed brand by Ophthalmologists<sup>1</sup>
- Preservative free: Contributes to comfort and tolerability<sup>2,3</sup>
- Now also available as Singules



**References:**

1. Cipla Data on File
2. Wagner RS, D'Arienzo PA, Hallas SJ, Thomson MB, Shapiro AM. A Comparative Study In a Normal Pediatric Population of the Relative Comfort of Moxifloxacin 0.5% Ophthalmic Solution versus a Tear Substitute. *Invest Ophthalmol Vis Sci* 2004; 45: E-Abstract 4936.
3. Katz et al. A fourth generation ocular fluoroquinolone, A suppl. To retractive care for Ophthalmologists. Vol. 7 No.3 March 2003

**"For any further information, please contact"**

**Cipla**

**Cipla Ltd. Regd, Office:**

Cipla House, Peninsula Business Park, Ganpatrao Kadam Marg,  
Lower Parel, Mumbai 400 013, INDIA  
Website: [www.cipla.com](http://www.cipla.com)

## *Contents*

---

<i>Editorial</i>	<i>1</i>
<i>Vital Dyes For Keratorefractive Surgeons</i>	<i>3</i>
<i>Nocardia Scleritis – Clinical Presentation and Management</i>	<i>8</i>
<i>Post Surface Ablation Haze</i>	<i>11</i>
<i>Resistance In Herpes Simplex and Varicella Zoster Viral Infections - A Brief Overview</i>	<i>16</i>
<i>Dosage and formulation of Antibiotics / Antifungals / Antiamoebics</i>	<i>20</i>
<i>Post LASIK Infective Keratitis</i>	<i>23</i>
<i>Types of Riboflavin &amp; Methods of Corneal Crosslinking</i>	<i>27</i>

---

# Editorial



aaa



**Rajesh Sinha**  
MD, DNB, FRCS  
Gen. Secretary ISCKRS

### Editorial Board

#### Editor in Chief

Rajesh Sinha

#### Associate Editor

Prafulla K. Maharana

#### Assistant Editor

Deepali Singhal

Manthan H. Chaniyara

Sridevi Nair

#### Editorial Committee

Vijay Sharma

Tarun Arora

Santosh Kumar

Srujana D

Sandeep Gupta

Ritika Sachdev

Namrata Sharma

Priya Tak

Rajib Mukherjee

Harshul Tak

Pallavi Sugandhi

Uma Sridhar

Sanjiv Mohan

Rajiv Kumar

#### Advisory Committee

Madan Mohan

G. Mukherjee

Jeewan S Titiyal

Virender Sangwan

Amar Agarwal

Samar Basak

Mahipal Sachdev

D Ramamurthy

Rishi Mohan

Ritu Arora

Radhika Tandon

M. Srinivasan

Ambarish Darak

Partha Biswas

Arun Jain

Ajay Dave

Umang Mathur

S. Bharti

Gaurav Luthra

Virendra Agarwal



INDIAN SOCIETY OF CORNEA  
AND KERATOREFRACTIVE SURGEONS

# KeraSight

Official Publication of Indian Society of Cornea and Keratorefractive Surgeons

## Executive Members



**Prof. Jeewan S Titiyal**  
President



**Dr. Rishi Mohan**  
Vice President



**Prof. Rajesh Sinha**  
General Secretary



**Dr. Rajib Mukerjee**  
Treasurer



**Prof. Namrata Sharma**  
Chairperson Scientific Committee



**Dr. Ritika Sachdev**  
Joint Secretary cum Joint  
Treasurer



**Dr. G. Mukherjee**  
Ex-Officio Member



**Dr. Ajay S Dave**  
Ex-Officio Member

# Vital Dyes For Keratorefractive Surgeons

## Vijay K Sharma, MS

Classified Specialist, Ophthalmology,  
Cornea and Refractive Surgery  
Army Hospital Research and Referral,  
Dhaura Kuan, New Delhi - 110010  
Email- vijaysharma007@gmail.com  
Phone- 091-8826956710



Lt Col  
Vijay K Sharma

## Introduction

Dyes are chemical compounds that bind to various substances in nature to induce colour. When dyes colour living tissues or cells, they are called vital dyes. Vital staining refers to the coloration of living cells or tissues. When vital staining is done in a living organism, it may be called intravital staining, whereas supravital staining is defined as the application of dyes to living cells or tissues freshly removed from the body. Vital dyes emerged as important and effective surgical adjuvants to enhance visualization of ocular tissues.

Commonly used vital dyes used by keratorefractive surgeons are as follows.

## Trypan Blue (TB)

TB is an anionic hydrophilic azo dye with the formula  $C_{34}H_{24}N_6Na_4O_{14}S_4$  and a molecular weight of 960 daltons. Azo dyes are large class of synthetic organic dyes that contain nitrogen in the azo form (-N 5 N-) in their molecular structures connecting aromatic ring compounds. Live cells/tissues with intact cell membranes usually are not coloured because their selective control of cellular membrane transport does not allow binding of TB. It has been widely used in both vitrectomy and cataract surgery and is commercially available in an 0.15% concentration for vitreoretinal surgery under the brand name Membrane Blue (DORC International, Zuidland, Netherlands) and as Vision Blue in a 0.06% concentration for cataract surgery (DORC International). TB as Membrane Blue and Vision Blue comes in a solution containing small amount of sodium salts, 8.2mg of NaCl, and water. The osmolarity of Vision Blue ranges from 257 to 314 mOsm/Kg and the pH from 7.3 to 7.6.

## USES

TB has been used in many eye banks to evaluate corneal endothelial cell viability. The blue dye stains the nuclei of damaged and dead endothelial cells in donor corneas, as well as areas of DM denuded

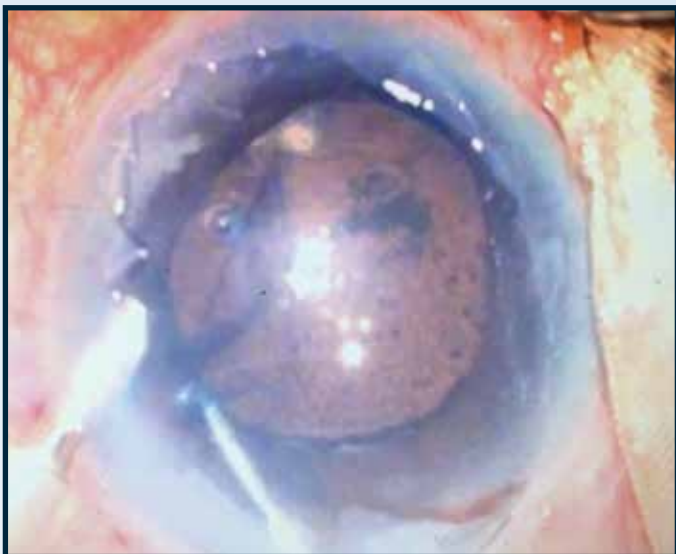
of endothelial cells. In order to assess endothelial damage and endothelial cell density by light microscopy, Sperling in 1986 introduced the use of TB diluted in 0.45% and 0.9% sodium chloride, or with 1.8% sucrose to induce dilation of intercellular spaces for visualization of endothelial cell borders. However, the reliability of TB-guided endothelial cell counts has been questioned. Although there was a significant correlation between the techniques, TUNEL-assay seemed to be more reliable in detecting earlier events in endothelial death process. At the frequently used concentrations of 0.001--0.1%, TB is not toxic to the corneal endothelial cells. Further investigation may clarify the benefits of TB-guided endothelial cell assessment for clinical practice.

TB is used in both penetrating and deep lamellar keratoplasties. For penetrating keratoplasty, 0.02% TB solution may be injected into the anterior chamber via a paracentesis to stain the DM of the donor as well as the recipient corneal tissue. Dye exposure may promote close alignment of edges of host and donor DM, thus improving graft stability and minimizing surgically induced astigmatism. TB may show both cut edges well, identify the corneal depth during suturing; and enable visibility of the viscosurgical device removal. Thereby the blue dye may improve tissue apposition.

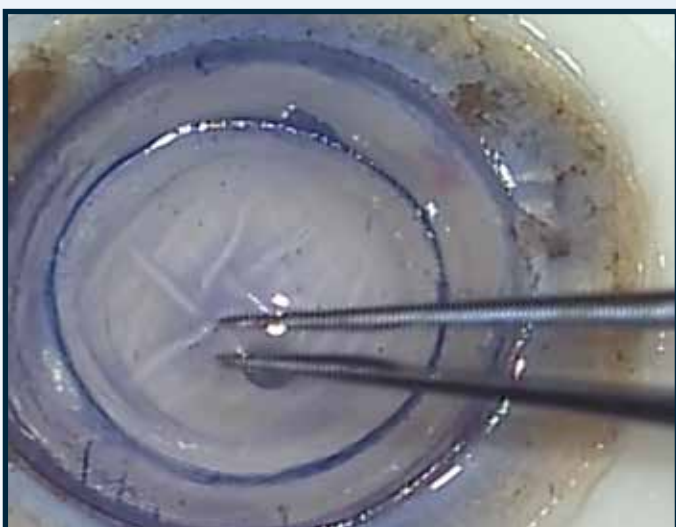
TB has been also used in order to facilitate stromal dissection and to avoid DM perforation in lamellar keratoplasty. After a two-thirds trephination of the cornea, 0.02% TB solution is injected intrastromally in four quadrants through a 30-gauge cannula for superficial dissection. Next, more TB is injected for deep dissection to stain the stromal fibers. This novel approach resulted in no residual stroma at the end of the intervention. TB disappeared totally in the early postoperative period.

Removal of DM during endothelial keratoplasty i.e. DMEK, DSAEK/ DSEK in pseudophakic eyes is greatly facilitated by staining the host DM with TB. (Fig 1&2)

It is also used to remove retained DM after penetrating keratoplasty as in congenital hereditary endothelial dystrophy because of longstanding stromal edema and loosening of the attachment. In this case, the simple, quick application of TB facilitates the removal of retained membranes during penetrating keratoplasty. In summary, the TB stain may be useful intra-operatively to visualize and remove the posterior stromal layers in modern keratoplasty.



**Figure 1.** Removal of trypan blue stained DM during descemetorhexis during DSAEK.



**Figure 2.** DMEK lenticule preparation using trypan blue.

Due to the frequent difficulty in finding clear corneal incisions during intraocular surgery, the use of TB-coated blades has been proposed. A 3.0-mm phaco incision blade tip was coated with TB in order to improve visualization. Fluid outflow and the mechanical effect of phaco tip movement during surgery also facilitated visualization of the incision, and no complications were reported with this surgical method. The vital dye had disappeared from the cornea after few days.

TB staining of the capsule edge in CCC was initially introduced in 1999. Since then the blue azo dye has been the most frequently used agent for staining the anterior capsule, as intra- operative injection of TB provides various advantages in cataract surgery. First, surgical TB injection may promote higher rates of success of capsulorhexis in phacoemulsification for cases with inadequate red reflexes. The rate of conversion to an extracapsular cataract extraction in white cataracts as the result of an incomplete CCC has been as low as 3.85% when TB is used, compared to 28.3% when no dye has been injected. Second, the blue vital stain allows staining of anterior and posterior capsules in children younger than 5, thereby enhancing effectiveness for completing the CCC. Thirdly, the importance of TB in the learning process of trainee surgeons and residents to perform CCC has been recently highlighted. Even in the presence of a good red reflex, young surgeons had a higher success rate of CCC when the dye was used. Finally, 0.3 ml of 0.06% TB has been applied to find the edge of a lost capsulorhexis, allowing the CCC completion.

The positive effect of TB staining of the lens capsule is that it may modify the biomechanical structure. Wollensak and co- authors recently performed a study on porcine eyes that evaluated 0.1% TB stain for 30 seconds, 1 minute, and 30 minutes with or without white light. TB staining for 1 and 30 minutes led to an increase in elastic stiffness and a significant decrease in ultimate extensibility in the anterior capsule. This effect was probably due to the photosensitizing action of TB to physical cross-linking of collagen through yielding of free oxygen radicals, which causes a change in elastic behavior.

Recently, Singh et al analyzed histological characteristics of TB-stained human capsules. They found out that TB mostly stained the basement membrane adjacent to the epithelial layer of the lens capsule with minimal lamellar staining in the

superficial basement membrane. Such findings may also explain why TB may provide differentiation between non-stained cortex and the lens capsule.

### Toxicity

Low doses of TB does not produce inflammation and corneal toxicity when injected into the anterior chamber. Clinically, TB is used in concentrations from 0.0125% to 0.4% to facilitate CCC. One uncontrolled case series where 0.1% TB was instilled into the anterior chamber found no effect on endothelial cell count or pachymetry during 8 years of follow-up. Another randomized clinical trial found 7.5% endothelial cell loss when 0.06% TB was placed under air bubble, compared to the 10.2% loss in the group with only air. In a rabbit model, TB showed no corneal endothelial cytotoxicity after 1minute exposure even at a concentration of 0.4%. TB placed under an air bubble or under viscoelastic produced no toxicity in the follow-up period.

Density and viability of lens epithelial cells (LEC) predicts posterior capsule opacification after cataract surgery. Nanavaty et al used 0.0125% TB to perform CCC in patients with white cataracts, and a sample of the anterior capsule was excised and compared with non-stained capsules. A significant decrease in LEC density was detected in eyes treated with TB compared with untreated ones, suggesting a toxic effect to the LEC. On the other hand, Melendez et al found neither toxicity nor photosensitization in cultured LEC with concentrations of TB ranging from 0.025 to 4mg/ml.

A relative contraindication for TB is the use of hydrophilic expandable acrylic intraocular lenses (IOLs), which have the highest water content (73.5%) of currently manufactured implants. This IOL is implanted dry, and its expansion depends on hydration by fluids in the capsular bag. Because the TB that remains in the aqueous humour may be absorbed into the lens, patients may experience more glare than with other IOLs; however, no significant difference was found in contrast sensitivity.

Bascal et al reported a case where an area of zonulysis allowed 0.06% TB to enter the vitreous cavity. They observed a moderate bluish hue of the vitreous that persisted until the fourth day. Multifocal ERG showed reduced responses which normalized after one month from the surgery, indicating a transitory retinal toxicity.

## Fluorescein

Fluorescein is a xanthene fluorophore with chemical structure of C<sub>20</sub>H<sub>12</sub>O<sub>5</sub> and molecular weight of 332 daltons. The vital dye in water has a very high fluorescence with maximal absorption at 494 nm and emission at 521 nm. Fluorescein may be conjugated with over 50 different salts or derivatives, including fluorescein sodium (FS) and fluorescein diacetate (FD). Fluorescein derivatives have been commonly used in microscopy, in dye laser as the gain medium, in serology to detect latent blood stains, and in dye tracing. Fluorescein is used extensively as a diagnostic tool, mainly as FS. (Fig 3)



*Figure 3. Fluorescein strips*

## Corneal Staining

Fluorescein doesn't penetrate intact corneal epithelium. But if there is a break in the epithelium, the fluorescein penetrates in to exposed Bowman's membrane and stroma and makes contact with alkaline interstitial fluid and turns in to bright green owing to its pH indicator properties. It is best examined under cobalt blue filter of the slit lamp and is very helpful in identifying the size of the occult lesions as well. Common staining patterns seen in the cornea are as follows

- Interpalpebral: dry eye
- Horizontal band across inferior 1/3 of cornea: lagophthalmos /exposure
- Superior punctate: SLK, floppy eyelid syndrome
- Central punctate: focal epithelial keratitis (Thygeson's SPK, epidemic keratoconjunctivitis, molluscum)
- Inferior punctate: blepharitis
- Perilimbal (360°): soft contact lens wear

**Seidel's test:** A drop of fluorescein is instilled into the lower fornix and patient is asked to blink to spread the dye evenly. On examination with slit lamp using cobalt-blue filter, fluorescein will be diluted by aqueous at the site of leakage



**Applanation tonometry:** After anaesthetizing the cornea with a drop of 2 per cent xylocaine and staining the tear film with fluorescein, patient is made to sit in front of slit-lamp. The cornea and biprisms are illuminated with cobalt blue light from the slit-lamp. Biprism is then advanced until it just touches the apex of cornea. At this point two fluorescent semicircles are viewed through the prism. Then, the applanation force against cornea is adjusted until the inner edges of the two semicircles just touch. This is the end point. The intraocular pressure is determined by multiplying the dial reading with ten.

**Contact lens fitting assessment:** Fluorescein allows us to evaluate the physical fit of a contact lens, or the bearing relationship that exists between a transparent contact lens and the eye. A yellow-green glow indicates the presence of fluorescein and therefore clearance between the lens and cornea. A deep blue or black area indicates the absence of fluorescein and therefore the lens is touching the cornea. Between these two extremes, the varying thickness of the tear layer is observed by varying shades of green. Bubbles indicate an air space and appear black.

**Tear film BUT:** It is the interval between a complete blink and appearance of first randomly distributed dry spot on the cornea. It is noted after instilling a drop of fluorescein and examining in a cobalt-blue light of a slit-lamp. BUT is an indicator of adequacy of mucin component of tears. Its normal values range from 15 to 35 seconds. Values less than 10 seconds imply an unstable tear film.

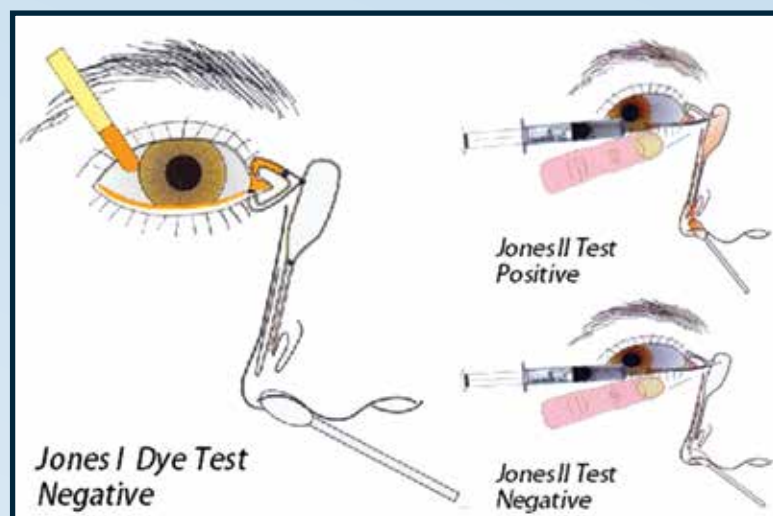
**FDDT:** Instill fluorescein in inferior fornix of both eyes, wait 5 minutes, then evaluate for asymmetric clearance of dye from tear meniscus. A prolonged retention of dye in conjunctival sac indicates inadequate drainage which may be due to atonia of sac or mechanical obstruction.

#### **Jones Dye Test:**

- **Jones I test:** Perform fluorescein dye disappearance test, then attempt to recover fluorescein in inferior nasal meatus with cotton-tipped applicator after 5 minutes; abnormal result (no fluorescein) occurs in 33% of normal individuals. If the dye is retained in cotton-tipped applicator, the cause of tearing

is probably hypersecretion i.e. epiphora.

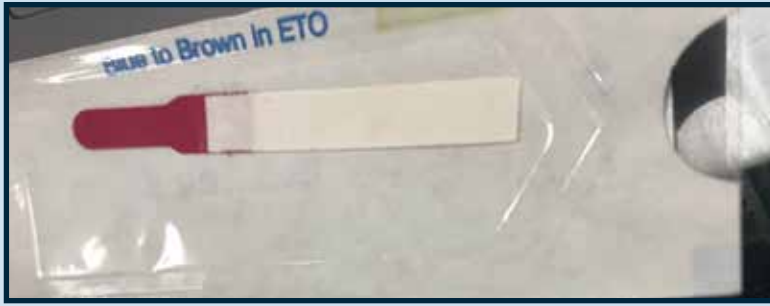
- **Jones II test:** After performing Jones I test, saline is irrigated into the nasolacrimal system; dye recovery from nose indicates functional occlusion of nasolacrimal duct; clear saline recovery indicates canalicular occlusion or nonfunctioning lacrimal pump. (Fig 4)



*Figure 4. Jones 1 and 2 tests.*

#### **Rose Bengal**

Rose Bengal (RB) is the 4,5,6,7-tetrachloro-2',4',5',7'-tetraiodo derivative of fluorescein and has been widely used to detect damage to the ocular surface epithelium (mainly on the conjunctiva, due to poor contrast on the cornea) (Fig. 5). Traditionally RB has been thought to stain dead or desquamated, but not healthy epithelial cells; this concept was introduced in the 1930s by Sjögren and later reiterated by Passmore and King, but no direct evidence was ever provided. In vitro studies on human corneal-limbal epithelial cells have demonstrated the important role of membrane-associated mucins (in addition to secreted mucins) in blocking RB staining. It is likely that in patients with dry eye RB staining occurs in areas lacking these mucins. Positive staining of the cornea with Rose Bengal indicates a qualitatively and/or quantitatively inadequate tear film. Hence this diagnostic staining is used particularly if there is suspicion of keratoconjunctivitis sicca or there is too rapid disruption of the tear film without epithelial defects (fluorescein-negative). The number of purplish-red flecks, which can only be counted carefully with the aid of a slit lamp microscope, is a measure of the loss of cells and consequently of the severity of the disturbance of the tear film.



*Figure 5. Rose Bengal strips*

This stain is irritating to the eye, so a local anesthetic should be applied in advance, and when defects are present the staining is very persistent. Feenstra and Tseng also highlighted the intrinsic toxicity of RB, which induces an instant loss of vitality (as shown by immediate cellular morphologic changes, subsequent loss of cellular motility, cell detachment and death). This intrinsic toxic effect is augmented by light exposure.

Rose Bengal has also been shown to stain the ocular surface in a variety of diseases that may not necessarily be associated with dry eye. In conjunctivochalasis RB staining can be observed along the redundant conjunctival folds, the area above these folds, the adjacent lid margin and the conjunctival non-exposure zone. In lipid layer of tear film deficiency that is caused by noninflamed meibomian gland dysfunction, RB staining preferentially occurs in the non-exposure zone, while in superior limbic keratoconjunctivitis it can be detected in the superior bulbar conjunctiva. In LASIK-induced neurotrophic epitheliopathy, RB staining typically affects the cornea. Finally, RB staining can be detected in atopic keratoconjunctivitis, herpes simplex keratitis and ocular surface neoplasia.

### **Lissamine green staining**

Lissamine green (LG) is a synthetic organic acid dye with two aminophenyl groups that stains the ocular

surface similar to RB without causing stinging. Staining is dose dependent and a minimal dose of 10–20  $\mu$ l is recommended, since inadequate volume results in a weak staining pattern that can be overlooked or underestimated. When LG is used, attention should be paid to the timing of observation, because an overly hasty evaluation is likely to hamper a full understanding of the staining pattern (evaluating the staining too quickly does not allow full development of the staining pattern and delay in evaluating the stain results in fading of the pattern). Ideally the grading should be performed between one and four minutes after staining. For a better reading it is also essential not to use an intense illumination beam, which may reduce the contrast leading to an underestimation of grading.

In the clinical setting, the staining profile of LG is nearly identical to that of RB and they are considered to be interchangeable. However, experimental studies have detected important differences between LG and RB: the former, for example, does not stain healthy corneal epithelial cells, nor does it affect their viability, whereas both characteristics are typical of RB.

### **Gentian Violet (GV)**

GV is used to mark the peripheral stromal surface containing DM and endothelium in cases of DSAEK/DSEK and DMEK. By identifying the side of the marked cornea, surgeons may insert the folded donor lenticule into the host anterior chamber in the proper position. This ensures that air is injected posterior to the graft, thus preventing the donor lenticule from unfolding upside down.

It is also used to mark the host cornea for proper centration before trephination during DALK and penetrating keratoplasty to aid in suturing.

# Nocardia Scleritis – Clinical Presentation and Management

**Rebika Dhiman, MD<sup>1</sup>; Sridevi Nair, MBBS<sup>1</sup>;  
Niranjana Nayak, MD<sup>2</sup>; Vanathi M, MD<sup>1</sup>; and  
Radhika Tandon, MD<sup>1</sup>**

<sup>1</sup> Cornea and Refractive Service, Dr. R.P Centre, All India Institute of Medical Sciences, New Delhi, India

<sup>2</sup> Ocular Microbiology Service, Dr. R.P Centre, All India Institute of Medical Sciences, New Delhi, India



Rebika Dhiman

## Corresponding author:

Dr Rebika Dhiman

Senior Resident Unit 6

Dr Rajendra Prasad Centre for Ophthalmic Sciences,  
AIIMS, New Delhi - 110029, India

Phone: +91-9910584535

Email: drrebika@gmail.com

Conflict of Interest and Source of Funding – None

**Keywords:** Nocardia, necrotizing scleritis, infectious scleritis, scleritis

## Abstract

**Aim:** To report an unusual case of Nocardia scleritis and present an updated review of literature.

**Methods & Results:** A 62 years old male diabetic patient was diagnosed with left eye necrotizing scleritis unresponsive to steroids. Microbiology revealed Nocardial infection. Prolonged and intensive medical management with topical fortified 2.5% amikacin and 0.5% moxifloxacin led to the resolution of scleritis.

**Conclusion:** A high index of suspicion of infection by the treating ophthalmologist and prolonged intensive medical management can significantly optimize the outcome in rare cases of Nocardia scleritis.

## Introduction

Necrotizing scleritis is a chronic destructive inflammatory disease of the sclera, which if left untreated can progress to perforation and possibly even blindness. In almost 40-90% of the patients of necrotizing scleritis there is an underlying autoimmune disease, Rheumatoid arthritis being the commonest of all [1-3].

Infectious scleritis constitutes just 5-10% of scleritis cases [1, 2, 4]. Nocardia is a rare cause of infectious scleritis. Nocardia keratitis and sclerokeratitis is commonly reported, but its presentation as an isolated scleritis has rarely been reported. We report this rare presentation of Nocardia scleritis and describe the various clinical presentations and management of ocular nocardial infections.

## Materials and Methods

A 62 years old male patient, farmer by profession, presented with chief complaints of gradually progressive, painful diminution of vision in the left eye for last two months associated with redness,

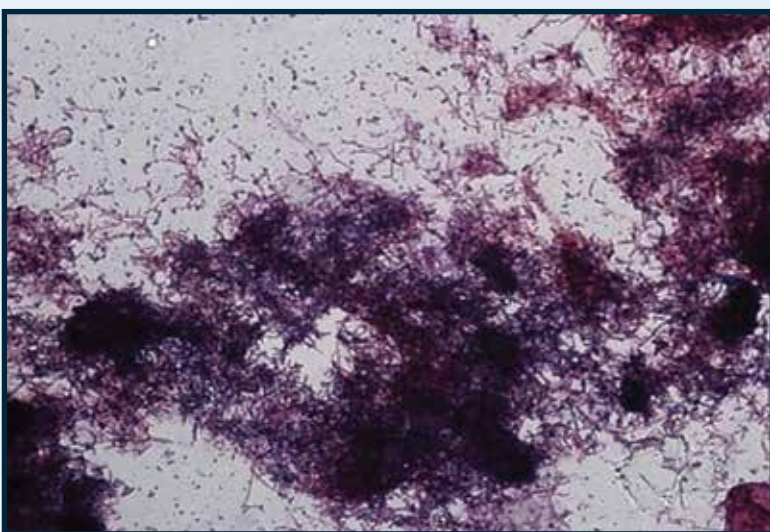
watering and headache. Patient was diabetic and on irregular oral hypoglycemic medications. He was diagnosed as a case of necrotizing scleritis by a private practitioner and medically treated with oral and topical steroids that resulted in deterioration of symptoms.

On examination, best-corrected visual acuity (BCVA) was 20/40 in both eyes. Ocular examination in left eye revealed two punched out inferior scleral lesions, measuring 4.5x2.8mm and 2.5x2mm respectively covered with whitish necrotic debris with underlying sclera. Two other similar lesions measuring 1x1.5mm were seen in the nasal and temporal sclera. Cornea was clear with a quiet anterior chamber. Posterior segment was normal in both the eyes (Figure 1).



**Figure 1.** Punched out sclera lesions with necrotic debris

Fasting blood sugar level was 151mg/dl. Serum biomarkers (for autoimmune diseases) were negative. Chest X-ray revealed a normal study. Scleral exudate was sent for microscopic examination (Gram stain, KOH stain, modified Ziehl-Neelsen stain using 1% H<sub>2</sub>SO<sub>4</sub>) and culture (blood agar and Sabouraud dextrose agar). Staining revealed thin, beaded, branching filaments that were gram positive and weakly acid fast. Culture on blood agar showed tiny, dry chalky white colonies suggestive of Nocardia species (Figure 2).



**Figure 2.** Thin beaded branching gram positive filaments on Gram's stain

Based on antibiotic sensitivity reports patient was started on topical amikacin 2.5% and topical

moxifloxacin 0.5% one hourly, topical cycloplegics 6 hourly and oral ciprofloxacin 500mg twice daily that was tapered over next three months.

## Results

After an initial response to treatment, patient had a recurrence of pain, diminution of vision (visual acuity of 20/200) associated with proptosis of 4mm (by Hertel's exophthalmometer) in the treating eye. Fundus was normal on examination. The patient was again started on frequent topical fortified antibiotics- 2.5% amikacin, 0.5% moxifloxacin and systemic antibiotics for a prolonged period of six months with a more gradual tapering to avoid any further recurrence. At six months, patient achieved BCVA of 20/40 with the healing of the lesion.

## Discussion

Infection constitutes a rare cause of necrotizing scleritis. Often the condition is associated with an underlying autoimmune disease that primarily requires treatment with steroids. In case an underlying infection is missed, as was seen in our case, this may worsen the disease. Hence, it is imperative to rule out infection in doubtful cases, and practice caution before starting steroids.

*Pseudomonas aeruginosa* is the most commonly reported organism worldwide in infectious scleritis [5-7] whereas in Indian scenario, *Aspergillus Flavus* and *Staphylococcus* have been reported to be the leading cause. *Nocardia* though a rare cause of scleritis worldwide, appears to be commoner in Indian scenario [8, 9]. This is possibly because of higher prevalence of *Nocardia* in Indian soil and greater risk of exposure to dust in an agricultural country like India [10]. The various predisposing factors in our patient were uncontrolled diabetes, agricultural profession that might have exposed the patient to some trivial trauma that he ignored and inappropriate steroid usage.

*Nocardia* is an aerobic, gram positive, weakly acid-fast, branching filamentous bacteria that belongs to the order Actinomycetales. A saprophyte in soil, *Nocardia* is not a part of normal ocular flora. Though systemic nocardiosis has been associated with immunocompromised status, ocular infections like keratitis, scleritis, orbital cellulitis, and endophthalmitis [11] have been commonly reported in immunocompetent individuals. Keratitis is the commonest ocular nocardial infections typically

presenting as a patchy anterior stromal infiltrates in a 'wreath' pattern or raised pinhead lesions. Scleritis usually occurs as an extension of keratitis with areas of well circumscribed punched out lesions [12]. Clinical presentation of our patient as isolated nocardia scleritis is rare.

The diagnosis is straightforward by staining and culture. But, availability of a microbiology set-up is a limiting factor in developing countries leading to a delay in diagnosis and subsequent complications like perforation. The management is mostly medical based on the culture and sensitivity reports. The sensitivity reported is 100% with topical fortified amikacin (2.5%) and topical trimethoprim (16mg/ml)-sulfamethoxazole (80mg/ml) and intermediate sensitivity for fluoroquinolones, cephalosporins and tobramycin. Amikacin is considered the drug of choice [13]. In our patient, the organism was sensitive to amikacin and moxifloxacin and responded to the same. Surgical intervention in the form of debridement may be needed along with the medical management [8]. A prolonged intensive treatment for atleast 4 to 6 months is essential for complete resolution and prevention of recurrences.

In conclusion, a high index of suspicion of infection by the treating ophthalmologist, avoiding inappropriate corticosteroid usage and a timely referral to a tertiary eye care center can significantly optimize the outcome even in rare cases of Nocardia scleritis.

## References:

1. Sainz de la Maza M, Jabbur NS, and Foster CS. Severity of scleritis and episcleritis. *Ophthalmology* 1994;101:389–396.
2. Akpek EK, Thorne JE, Qazi FA et al. Evaluation of patients with scleritis for systemic disease. *Ophthalmology* 2004;111:501–506.
3. Okhravi N, Odufuwa B, McCluskey P et al. Scleritis. *Surv Ophthalmol* 2005;50:351–363.
4. Jabs DA, Mudun A, Dunn JP et al. Episcleritis and Scleritis-clinical features and treatment results. *Am J Ophthalmol* 2000;130:469–476.
5. Lin CP, Shih MH, Tsai MC. Clinical experience of infections scleral ulceration-a complication of pterygium operation. *Br J Ophthalmol* 1997;81:980–983.
6. Hsiao CH, Chen JJ, Huang SC, et al. Intrasceral dissemination of infectious scleritis following pterygium excision. *Br J Ophthalmol* 1998;82:29–34.
7. Huang FC, Huang SP, Tseng SH. Management of infections scleritis after pterygium excision. *Cornea* 2000;19:34–39.
8. Jain V, Garg P, and Sharma S. Microbial scleritis -experience from a developing country. *Eye* 2009;23:255–261.
9. Kumar Sahu S, Das S, Sharma S et al. Clinico-Microbiological Profile and Treatment Outcome of Infectious Scleritis: Experience from a Tertiary Eye Care Center of India. *Int J Inflam* 2012;753560.
10. Haripriya A, Lalitha P, Mathen M et al. Nocardia endophthalmitis after cataract surgery: clinicomicrobiological study. *Am J Ophthalmol* 2005;139:837–846.
11. Decroos FC, Garg P, Reddy AK et al. Hyderabad Endophthalmitis Research Group. Optimizing diagnosis and management of nocardia keratitis, scleritis, and endophthalmitis: 11-year microbial and clinical overview. *Ophthalmology* 2011;118:1193–120.
12. Sridhar MS, Gopinathan U, Garg P et al. Ocular nocardia infections with special emphasis on the cornea. *Surv Ophthalmol* 2001;45:361-78.
13. Reddy AK, Garg P, Kaur I. Speciation and susceptibility of Nocardia isolated from ocular infections. *Clin Microbiol Infect* 2010;16:1168-71.

## Post Surface Ablation Haze

**Lt. Col. Santosh Kumar, MS;  
Rajesh Sinha, MD, DNB, FRCS**

R P Centre, AIIMS

### Corresponding author:

Dr Rebika Dhiman

Senior Resident Unit 6

Dr Rajendra Prasad Centre for Ophthalmic Sciences,

AIIMS, New Delhi - 110029, India

Phone: +91-9910584535



**Lt. Col. Santosh  
Kumar**

### Introduction

Refractive Surgeries like Photorefractive Keratectomy (PRK), Laser-assisted Subepithelial Keratomileusis (LASEK) and Epi-Laser in situ Keratomileusis (Epi LASIK) have one thing in common that they all use surface ablation in some form and this may be the reason for post-operative corneal haze. In all the three procedures, laser ablation with excimer laser is done after removal of corneal epithelium but with different techniques. In PRK, epithelium is removed mechanically with micro instruments, in LASEK it is ethanol-assisted and in Epi-LASIK epikeratome is used to separate epithelium. In LASEK and Epi-LASIK epithelium is repositioned back after ablating corneal stroma but in PRK epithelial healing occurs by primary intention as there is no epithelial flap. This may be the reason that post-operative haze is mostly seen with PRK (Figure 1). Subepithelial haze is seen almost in all post PRK patients after 1month, reaching the greatest intensity at 3-6 months, which gradually decreases from there.<sup>1,2,3</sup>

Commonest risk factors accounting for increased post-operative corneal haze are:

- Myopic correction > -6.0D, Hyperopic correction > +3.5D, Astigmatism > 1.5D
- Ablation depth > 80 $\mu$ m
- Smaller diameter ablation profile
- Retreatment / enhancement
- Previous corneal surgery

Other factors that may influence occurrence of sub-epithelial haze

following surface ablation includes pregnancy, oral contraceptive use, ultraviolet (UV) radiation exposure, ocular surface disorders and those with brown irides.<sup>4,5</sup>

### Historical Perspective and Epidemiology

The advent of Excimer laser brought the origin of corneal refractive procedures. PRK was developed by Trokel and colleagues in 1983 using excimer laser that emits ultraviolet light of 193 nm. In 1996, it was approved by Food and Drug Administration (FDA) as refractive surgery technique.<sup>6,7</sup> Since then thousands

of people have undergone PRK World over. The PRK had limitations of post-operative discomforts in the form of pain and corneal haze. PRK was followed by LASIK which had excellent results without post-operative discomforts and with immediate visual recovery with 'wow' factor. LASIK has its own share of complications in the form of post-operative corneal ectasia and flap complications which led to resurgence of flap less refractive surgeries i.e.



*Figure 1a. Punched out sclera lesions with necrotic debris*

surface ablation. The prevalence of post-operative corneal haze has been studied in different studies. It is variable and depends on pre-operative risk factors and the surgical approach. In a study by Netto et al the incidence of clinically significant corneal haze was 0.3% to 3%.<sup>8</sup> In another study at Singapore by Bryan chin et al, it was reported to be around 2.3 % and it was more when refractive surgery was done for higher corrections.<sup>9</sup>

## Pathophysiology

Post surface ablation haze has been explained by exaggerated corneal healing response in the form of abnormal tissue reaction. The corneal healing process begins following epithelial injury with the release of inflammatory mediators. Activated and upregulated growth factors, cytokines and chemokines produced by epithelial cells and found in the tear film contribute to wound healing and injury response. In the absence of protective epithelial barrier, epithelial-stromal tissue interaction initiates a cascade of events leading to keratocyte apoptosis and necrosis. This is followed by proliferation of remaining keratocytes and they synthesize new extracellular matrix which causes corneal haze. These keratocytes migrate to repopulate stroma, in turn activating fibroblasts and causing myofibroblast formation. These myofibroblasts are formed in response to Transforming growth factor beta (TGF  $\beta$ ) signal pathway, which is the tissue response pathway after any penetrating injury. Myofibroblasts have greater reflectivity and tend to be less transparent because of altered crystallin production. Their extracellular matrix exhibits less organization and increased density which causes increased light scattering and haze formation.<sup>10</sup>

In refractive surgeries with surface ablation, there is loss of epithelial basement membrane, more so in PRK, which is responsible for corneal haze post operatively. The epithelial basement membrane is believed to act as a barrier between the stromal keratocytes and the inflammatory molecules released from the tear film and epithelium. Loss of this causes increased direct exposure of stromal tissue to the inflammatory mediators released from epithelium and tear film.

Another issue with PRK is irregular surface, which has also been related to high incidence of haze. Increased dioptric corrections results in more surface irregularity, which results in increased corneal haze.

Interestingly, surface irregularity has been positively correlated with increased myofibroblasts in anterior stromal tissue.<sup>11</sup> The mismatch of plasminogen activator- plasmin also accounts for the corneal haze due to accumulation of extracellular matrix.

## Clinical Symptomatology

Post-operative corneal haze generally causes fluctuation of vision, irregular astigmatism, reduction in contrast sensitivity, difficult night vision, myopic regression and reduction in best-corrected visual acuity (BCVA).

## Types of Haze

Based on symptomatology, 2 types of corneal haze have been observed.<sup>8</sup> The more common is transitory haze, which is found between 1 and 3 months post operatively and rarely with clinical symptoms. This resolves mostly within 1 year. Another type is late onset corneal haze which occurs between 2 and 5 months after surgery and can cause significant compromised vision, myopic regression and can last up to 3 years.<sup>1,12</sup> This is generally associated with high myopic ablations and rarely can cause permanent changes requiring surgical treatment.

Corneal haze can be quantified objectively as well as subjectively. Objective grading can be done through various instruments noting the degree as well as depth of the corneal haze. For this confocal microscopy and scheinplflug densitometry is used.<sup>13</sup> Anterior segment optical coherence tomography (ASOCT) can also be used to measure the haze when surgical management is planned.

Subjective grading can be done based on slit lamp findings. Various investigators have devised their grading based on clinical details noted. Fantes and colleagues described 5 stages of haze based on visualization of the iris/lens details with completely clear cornea graded as grade 0 and opacification of stroma with entirely obscured view of anterior chamber graded as grade 4.<sup>14</sup> Similar grading system has been given by Hanna et al based-on visualization of iris details by slit lamp.<sup>15</sup>

## Management

Management of post surface ablation haze includes prevention, medical treatment and surgery. Prevention is aimed at decreasing corneal stromal inflammation as well as fibrosis. As there are more

chances of corneal haze after higher ablations, the role of prevention comes into play in these cases. Various modalities of preventive techniques have been described in the literature. Out of which, most practiced one is use of intra-operative Mitomycin C (MMC) and post-operative topical steroids.

MMC is an antineoplastic antibiotic agent of the family of anti-tumour quinolones, derived from *Streptomyces caespitosus*. It is a potent DNA crosslinker and acts in a dose dependent manner. MMC inhibit cell mitosis, including epithelial and stromal cells. MMC is used widely in other ophthalmic conditions like conjunctival neoplasms, pterygium and glaucoma surgeries to prevent fibrosis, scarring and neoplasm recurrence. It is generally used prophylactically in high risk cases which are known to have post ablation corneal haze i.e. high myopia and deeper ablations more than 80 $\mu$ m. The concentration of MMC, used is 0.02% and is applied for a duration of 30 seconds to 2 minutes after ablation.<sup>3,6,16</sup>

Although the application of MMC is beneficial for corneal recovery, it is necessary to control the doses and the time of exposure. Thornton et al, in one of studies showed that concentration is a more important factor than the duration of MMC exposure in corneal haze prevention. He advocates that for high myopia corrections (>-6.00D) standard concentration of topical MMC (0.02%) may be used, whereas for moderate myopia (-3.00 to -5.90D) low dose of MMC (0.002%) may be considered.<sup>17</sup>

Rajan et al analyzed the effects of MMC after correction of -9.00D by PRK in 3 groups: without MMC application, with MMC (0.2mg/ml) application for 1min and with MMC (0.2mg/ml) application for 2 min. The 2 min MMC group had thinner epithelium than the 1min and without MMC application groups.<sup>18</sup>

Fazel et al found that two-step administration of 0.02% MMC (45 seconds, followed by 15 seconds) further decreased corneal haze formation in high myopia, compared to a single dose of 45 seconds.<sup>19</sup> Shojaei et al found that short-time MMC exposure prevents low-grade haze in low ablation depths. The MMC doses oscillate between 0.002% and 0.06%. The intraoperative application of 0.02% MMC solution is the most recommended, as it produces less corneal haze, and provides better uncorrected visual acuity and best spectacle-corrected visual acuity.<sup>20</sup>

In one of the studies by Majumdar et al, a prospective randomized trial, 60 eyes were included for ablation with refractive error ranging from -6.00D to -10.00D. MMC treated eyes had minimal haze whereas control eyes had significant haze in almost 63% of patients.<sup>21</sup> Mitomycin C was used in dose of 0.02% for 2 minutes. Few studies have compared the effectivity of MMC in dosage of 0.02% versus 0.01% in moderate myopia. Ramjoo et al found similar refractive and haze outcomes with 0.01% and 0.02% MMC for moderate myopia, recommending the use of 0.01%.<sup>22</sup>

Though MMC is effective in preventing post ablation haze, its use is not without complications. Various complications have been reported in literature i.e. ocular surface toxicity including stem cell deficiency, sterile melting and delayed epithelialization.<sup>23</sup> Although unusual, scleral ulceration and non-healing conjunctival defects have also been reported with high MMC doses (0.04%). As MMC is applied in the stromal bed, it seems that it might penetrate the anterior chamber, because cytotoxic effects on the ciliary body epithelium have been reported.<sup>24</sup> There has been reports of toxic effect of MMC in the overall morphology of the endothelium. Morales et al found that intraoperative 0.02% MMC applied for 30 seconds after PRK induced corneal endothelial cell loss.<sup>25</sup> Diakonis et al applied MMC for 15 seconds and the density of endothelial cells was not affected.<sup>24</sup>

Because of these potential complications, use of MMC should be restricted to the central cornea and surface of the eye and fornices should be thoroughly irrigated following application. Apart from use of MMC, other agents have been used to prevent post ablation haze. Use of topical steroid within first 72 hours is supposed to prevent haze formation.<sup>1</sup> For this purpose, topical prednisolone or soft steroid i.e. lotoprednol or fluorometholone has been used, however it should not be used for longer duration to curtail the risk of steroid responders. Non-steroidal anti-inflammatory drugs (NSAID) have also been used in place of steroid in few studies but results are not as good as steroids.<sup>6</sup>

Use of high dose vitamin A (25000 IU) and vitamin E has been suggested in initial 3 months post ablation to decrease the incidence of haze. They promote reepithelialization by reduction of free oxygen radicals. Prophylactic use of Vitamin C (ascorbic acid) in dosage of 1000 mg daily has been studied



to prevent haze as they are supposed to reduce keratocyte activation, impede UV damage and prevent formation of free oxygen radical.<sup>1</sup>

New modalities of treatment are being investigated for treatment of corneal haze. Role of plasma rich in growth factors (PRGF-Endoret) has been investigated and has shown good results.<sup>26</sup> Another promising result has been seen with topical interferon alpha-2b which has anti-scarring effects.<sup>27</sup> There is definitive role of TGF-beta in causing post-operative haze and to counter act its effect, role of anti-TGF drugs i.e. Trichostatin A is being investigated.<sup>6</sup> There is proven role of TGF beta 1 and TGF beta 3 in corneal wound healing. TGF beta 1 promotes scarring and beta 3 inhibits it. Use of TGF beta 3 to inhibit the action of TGF beta 1 is being attempted in in-vitro model to prevent the occurrence of sub-epithelial haze following surface ablation. Another treatment still in experimental stage is gene therapy in the form of Targeted AAV5-Smad 7 gene therapy.<sup>28</sup>

Theoretically there is a role of amniotic membrane after PRK ablation to decrease the haze formation as it has anti-inflammatory as well as anti-scarring properties but it seems an impractical solution as post-operative vision will be grossly decreased with use of amniotic membrane. Another modality suggested is trans-epithelial ablation, role of which is yet to be proved.

Surgical treatment should be resorted to if patient is not responding to these modalities and if corneal scar has become permanent in nature. In these cases, superficial debridement with application of topical MMC followed by polishing with burr has been suggested. The role of Phototherapeutic keratectomy (PTK) along with MMC in these cases has been suggested. If nothing works, then sutureless superficial anterior lamellar keratoplasty may be needed, although in very few cases, to restore the visual acuity and clarity of cornea.

## References

1. Jordan A Margo, Wuqaas M Munir. Corneal haze following refractive surgery: A review of pathophysiology, incidence, prevention, and treatment. *International Ophthalmology Clinics*.2016; 56: 111-125.
2. Carones F, Vigo L, Carones AV, et al. Evaluation of photorefractive keratectomy retreatments after regressed myopic laser in situ keratomileusis. *Ophthalmology* 2001;108:1732-1737.
3. Braunstein RE, Jain S, McCally RL, et al. Objective measurement of corneal light scattering after excimer laser keratectomy. *Ophthalmology*1996;103:439-443.
4. Bilgihan k, Ozdek SC, Akata F, et al. Photorefractive keratectomy for post penetrating keratoplasty myopia and astigmatism. *J Cataract Refract Surg*. 2002; 26:1590-1595.
5. Taylor SM, Fields CR, Barker FM, et al. Effect of depth upon the smoothness of excimer laser corneal ablation. *Optom Vis Sci*. 1994; 71:104-108.
6. Corneal regeneration after photorefractive keratectomy: A review. *Journal of Optometry*. 2015; 8:149-169.
7. Trokel SL, Srinivasan R, Braren B. Excimer laser surgery of the cornea. *Am J Ophthalmol*. 1983; 96:710-715.
8. Netto MV, Mohan RR, Ambrosio R, et al. Wound healing in the cornea: a review of refractive surgery complications and new prospects for therapy. *Cornea*. 2005; 24: 509-522.
9. Risk factors for early-onset corneal haze after photorefractive keratectomy in an Asian population. *J Cataract Refract Surg* 2016; 42: 710-716.
10. Wilson SE, Liu JJ, Mohan RR. Stromal- epithelial interactions in the cornea. *Prog Retin Eye Res*. 1999; 18: 293-309.
11. Netto MV, Mohan RR, Sinha S, et al. Stromal haze, myofibroblasts, and surface irregularity after PRK. *Exp Eye Res*. 2006; 82: 788-797.
12. Lipshitz I, Loewenstein A, Varssano D, et al. Late onset corneal haze after photorefractive keratectomy for moderate and high myopia. *Ophthalmology* 1997; 104:369-373.
13. Tavakoli M, Hossain P, Malik RA. Clinical applications of corneal confocal microscopy. *Clin Ophthalmol Auckl NZ*. 2008; 2:435-445.
14. Fantes FE, Khalil HD, Waring GO, et al. Wound healing after excimer laser Keratomileusis (photorefractive keratectomy) in monkeys. *Arch Ophthalmol*. 1990;108:665-675.
15. Hanna KD, Pouliquen YM, Waring GO, et al. Corneal wound healing in monkeys after repeated excimer laser photorefractive keratectomy. *Arch Ophthalmol*. 1992; 110:1286-1291.
16. Yamamoto T, Varani J, Soong HK, et al. Effects of 5-FU and mitomycin C on cultured rabbit subconjunctival fibroblasts. *Ophthalmology* 1990; 97: 1204-1210.
17. Thornton, I, Xu M, Krueger RR. Comparison of standard (0.02%) and low dose (0.002%) mitomycin C in the prevention of corneal haze following surface ablation for myopia. *J Refract Surg*. 2008; 24:568-576.
18. Rajan MS, O'Brart DP, Patmore A, Marshall J. Cellular effects of mitomycin-C on human corneas after photorefractive keratectomy. *J Cataract Refract Surg*. 2006;32: 1741-1747.
19. Fazel F, Roshani L, Rezaei L. Two-step versus single application of mitomycin-C in photorefractive keratectomy for high myopia. *J Ophthalmic Vis Res*. 2012; 7:17-23.
20. Shojaei A, Ramezanzadeh M, Soleyman-Jahi S, Almasi-Nasrabadi M, Rezazadeh P, Eslani M. Short-time mitomycin-C application during photorefractive keratectomy in patients with low myopia. *J Cataract Refract Surg*.2013; 39:197-203.

21. Majumdar PA, Schallhorn SC, Cason JB, et al. Mitomycin-C in corneal surface excimer laser ablation techniques: a report by the American Academy of Ophthalmology. *Ophthalmology* 2015; 122:1085-1095.
22. Razmjoo H, Kooshanmehr Mr, Peyman A. Comparison of standard and low dose intraoperative mitomycin C in prevention of corneal haze after photorefractive keratectomy. *Int J Prev Med.* 2013;4:20.
23. Hardten DR, Samuelson TW. Ocular toxicity of mitomycin-C. *Int Ophthalmol Clin.* 1999;39:79-90.
24. Diakonis VF, Pallikaris A, Kymionis GD. Alterations in endothelial cell density after photorefractive keratectomy with adjuvant mitomycin. *Am J Ophthalmol.* 2007; 144:99-103.
25. Morales AJ, Zadok D, Mora-Retana R, Martinez-Gama E, Robledo NE, Chayet AS. Intraoperative mitomycin and corneal endothelium after photorefractive keratectomy. *Am J Ophthalmol.* 2006;142:400-404.
26. Anitua E, Muruzabal F, Alcalde I. Plasma rich in growth factors (PRGF-Endoret) stimulates corneal wound healing and reduces haze formation after PRK surgery. *Exp Eye Res* 2013; 115:153-61.
27. Gillies MC, Garrett SK, Shina SM. Topical interferon alpha 2b for corneal haze after excimer laser photorefractive keratectomy. The Melbourne Excimer Laser Group. *J Cataract Refract Surg;* 1996;22(7):891-900.
28. Gupta S, Rodier JT, Sharma A, Giuliano EA, Sinha PR, Hesemann NP, et al. Targeted AAV5-smad 7 gene therapy inhibits corneal scarring in vivo. *PLoS ONE* 2017; 24;12(3):e0172928.

# Resistance In Herpes Simplex and Varicella Zoster Viral Infections - A Brief Overview

**Dr. Aditya Pradhan, DNB, MNAMS, FICO**

Consultant

Department of Cornea and External Disease

Disha Eye Hospitals, Kolkata



**Aditya Pradhan**

## Introduction

Herpes simplex viruses 1 (HSV-1) and 2 (HSV-2) cause orolabial and genital infections as well as keratitis, encephalitis and neonatal infections. Varicella-zoster virus (VZV) is the causative agent of varicella and herpes zoster. Farooq et al have estimated the incidence of HSV keratitis in developed nations to be around **242000 cases/year** and to be **1-1.5 million cases/year** in developing nations<sup>1</sup>. There are no epidemiological studies available from India regarding the same.

Viral DNA polymerase (pol), as well as viral thymidine kinase (TK), is one of the early proteins produced in infection and is responsible for chain elongation. It is the target for antiviral drugs such as acyclovir, ganciclovir, famciclovir, valacyclovir, foscarnet and cidofovir, which all have activity against HSV. **All antiviral agents target the viral DNA pol<sup>2</sup>.**

The first-line drugs include deoxy-guanosine analogues such as acyclovir (ACV) and their L-valyl-ester prodrugs valaciclovir (VACV) and famciclovir (FCV). ACV and penciclovir (PCV) must be phosphorylated by the thymidine kinase of HSV or VZV and then by cellular kinases to be converted into their active metabolites. Their triphosphate forms compete with deoxynucleotide triphosphates (dNTPs) for incorporation into the replicating DNA chain. In addition, ACV triphosphate acts as a DNA chain terminator.

The second-line drug is the pyrophosphate analogue foscarnet (FOS) that is administered intravenously for the treatment of nucleoside analogue-resistant HSV and VZV infections. **FOS does not need any activation step to exert its antiviral activity** and thus directly binds to and blocks the pyrophosphate-binding site on the viral DNA pol, which prevents incorporation of incoming dNTPs into viral DNA. Topical and intravenous formulations of cidofovir (CDV) may be used 'off label' in the treatment of nucleoside analogue resistant and/or FOS-resistant HSV infections. CDV is an acyclic nucleoside phosphonate that only requires phosphorylation by cellular enzymes to be activated. Once in its diphosphate form, CDV inhibits the viral DNA pol by acting as a DNA chain terminator.

## Emergence of resistance

In immunocompetent individuals, the prevalence of HSV isolates resistant to ACV ranges from 0.1-0.7%<sup>3</sup>. In immunocompetent patients with herpetic keratitis, the prevalence is around 6.4%<sup>4</sup>. Resistance is the most in immunocompromised patients at around 3.5-14% and as high as 36% in haematopoietic stem cell transplant recipients<sup>5</sup>. The picture is not so clear as regards to VZV isolates resistant to ACV with only a few reports available.

## Mechanisms of resistance

In HSV clinical isolates, resistance to ACV is mediated in 95% of the cases by mutations in the viral TK and, in the remaining cases, by mutations in the viral DNA pol<sup>3,5</sup>. In VZV clinical isolates, resistance to ACV is mostly conferred by mutations in the viral TK and, less frequently, by mutations in the viral DNA pol<sup>5</sup>. Mutations in TK account for the majority of cases with ACV resistance<sup>4,6</sup>. Patients with ACV resistant strains of HSV caused by a TK mutation are also resistant

to VACV, ganciclovir, and FCV because these antiviral agents also rely on TK phosphorylation. Antiviral agents that do not rely on TK phosphorylation (FOS, CDV, and trifluridine) will not share cross-resistance to acyclovir when there is a TK mutation.

## Evaluation of HSV and VZV drug susceptibility

There are phenotypic and genotypic assays available to measure drug susceptibility and resistance. Levels of drug resistance (EC50 values) for HSV are best measured by cell-based (phenotypic) assays. The gold standard phenotypic method to determine the susceptibility of HSV isolates to antiviral drugs is the plaque reduction assay (PRA) in Vero cells that is approved as a standard protocol by the Clinical and Laboratory Standards Institute<sup>7</sup>.

Susceptibility of VZV to antiviral drugs can be tested by the PRA using fibroblastic cells<sup>8</sup>. However, the clinical utility of the PRA is limited by the low rate of VZV isolation most exclusively from vesicle fluids and its slow growth in cell culture (typically 5–6 days)<sup>9</sup>.

Genotypic assays are too complex to describe in this overview and the interested reader can refer to the excellent review article by Piret et al<sup>A</sup>.

## Resistance in the Indian scenario

Pramod et al analysed seven HSV-1 isolates from keratitis cases which were clinically resistant to ACV. 4 out of the 7 isolates proved ACV resistant by PRA and 3 of them showed reduced TK activity<sup>10</sup>.

Abraham et al undertook a cross-sectional study to determine the ACV susceptibility of HSV- 1 & 2 isolates using a dye uptake assay. 130 isolates from 141 patients were analysed and the overall prevalence was found to be 5.4% (3% in HSV-1 and 7.8% in HSV-2)<sup>11</sup>.

## Risk factors

Long-term prophylaxis in the form of oral ACV has been implicated as a risk factor for resistance. However, the evidence is conflicting. In a study of isolates from 239 patients who had previously been on suppressive ACV treatment for 6 years, the median ACV sensitivity was not statistically significantly different from that of ACV-naïve patients<sup>12</sup>. A similar study found no significant difference in the incidence of ACV resistance between patients who previously received ACV treatment and treatment-

naïve immunocompetent patients (0.67% vs. 0.42%, respectively)<sup>13</sup>.

The prevalence of ACV-resistant HSV isolates specifically in ocular infections was assessed in two studies. The first analysed 40 HSV-1 isolates from 35 patients using a dye uptake method and found that one isolate was resistant whereas three had reduced sensitivity. Thirteen of the cases had keratitis<sup>14</sup>. In a second study of 173 immunocompetent patients with HSV keratitis, 11 (6.4%) had ACV-resistant isolates. Ten of the 11 had mutations in the viral TK gene conferring the resistant phenotype. Interestingly, one isolate had cross-resistance to foscarnet as well<sup>4</sup>.

Van Velzen et al in an elegant study showed that long-term ACV prophylaxis ( $\geq 12$  months) predisposes to ACV-refractory disease due to the emergence of corneal ACV resistant HSV-1. Recurrence duration of  $\geq 45$  days was also identified as an independent risk factor<sup>15</sup>.

The high incidence of ACV-resistance in this population of patients can be explained because the cornea can be regarded as an immune-privileged site, with low immune surveillance allowing for rapid selection of mutant viruses. Furthermore, the same ACV-resistant virus could be recovered from the cornea during consecutive herpetic keratitis episodes, indicating that the ACV-resistant HSV-1 is able to establish latency and reactivate<sup>15</sup>.

A recent report demonstrated the coexistence of ACV-sensitive and ACV-resistant latent HSV-1 in the trigeminal ganglia from immunocompetent individuals<sup>16</sup>.

## Role of PCR

Polymerase chain reaction (PCR) is a valuable tool in the diagnosis of HSV/VZV keratitis. A real-time polymerase chain reaction (Real-Time PCR), also known as quantitative polymerase chain reaction (qPCR), is a laboratory technique of molecular biology based on the PCR. It monitors the amplification of a targeted DNA molecule during the PCR, i.e. in real-time, and not at its end, as in conventional PCR. Real-time PCR can be used quantitatively (quantitative real-time PCR), and semi-quantitatively, i.e. above/below a certain amount of DNA molecules (semi quantitative real-time PCR).

Pramod et al studied the utility of PCR analysis

against other diagnostic markers: HSV isolation on cell culture, HSV antigen detection by indirect immunofluorescence, detection of anti-HSV IgG by enzyme-linked immunosorbent assay (ELISA) and detection of HSV-specific tear secretory IgA (sIgA) by ELISA. These tests showed overall sensitivity values of 22.4%, 39.8%, 30.4% and 20.3% respectively<sup>17</sup>.

Hlinomazová et al found that the sensitivity of RT-PCR for detection of HSV-1 infection was higher than 90%. They also diagnosed 16 cases of ACV resistant keratitis<sup>18</sup>.

Encouraging results regarding the utility of RT-PCR were also reported by Aliabadi et al and Ma et al<sup>19,20</sup>.

Kowalski et al reported the results of a new test called AmpliVue that can detect HSV-1/2 DNA and provide results within 1 hour. They found that the sensitivity of AmpliVue against ocular samples that were both culture-positive and PCR-positive was 84%. The specificity of AmpliVue was 100%<sup>21</sup>.

## Management

The persistence of active lesions due to HSV (not necessarily keratitis) for more than 7–10 days after initiation of high-dose oral ACV, VACV or FCV therapy without apparent decrease in size, an atypical appearance or the emergence of satellite lesions is suggestive of treatment failure. The persistence of clinical signs of VZV infection for more than 10–14 days after initiation of ACV therapy is suggestive of treatment failure. An algorithm is given below<sup>A</sup>.

## Novel strategies and the road ahead

Some promising compounds are currently in clinical trials. The orally bioavailable lipid ester prodrug of CDV (i.e., hexadecyloxypropyl-cidofovir; brincidofovir) could avoid the dose-limiting toxicity of the parent drug and provide a safe alternative for ACV-resistant herpes viruses in immune-compromised patients<sup>22</sup>. Pritelivir, a potent orally bioavailable helicase primase inhibitor, reduced the rates of genital HSV-2 shedding and days with lesions in a phase II trial<sup>23</sup>. The bicyclic nucleoside analogue FV-100 and carboxylic nucleoside analogue valomaciclovir were well tolerated and effective for the treatment of herpes zoster in phase II trials<sup>24,25</sup>. Novel classes of antiviral agents targeting the ribonucleotide reductase, the helicase-primase complex and the process of viral DNA encapsidation are at earlier stages of development<sup>25</sup>.

## Conclusion

Resistance of HSV to ACV is a well-established problem which hasn't been given due importance in light of emerging resistance to antibacterials and antifungals. Surveillance programs elsewhere are targeted towards bacteria and don't exist in our country. The treating clinician must have a logical approach towards infectious keratitis. There should be a high index of suspicion in cases which are bilateral, which are immune-compromised, in recurrent HSVK and those showing poor response to treatment. Long term prophylaxis with ACV should be in selected cases and not routinely although it seems inevitable in certain scenarios like post corneal transplantation<sup>B</sup>.

## References

1. Farooq A, Shukla D. Herpes Simplex Epithelial and Stromal Keratitis: An Epidemiologic Update. *Surv Ophthalmol.* 2012;57(5):448-462.
2. Andrei G, de Clercq E, Snoeck R. Viral DNA polymerase inhibitors. In: Cameron CE, Gotte M, Raney K, editors. *Viral genome replication.* New York: Springer; 2009. pp. 481–526.
3. Piret J, Boivin G. Resistance of herpes simplex viruses to nucleoside analogues: mechanisms, prevalence, and management. *Antimicrob Agents Chemother.* 2011; 55:459–472.
4. Duan R, de Vries RD, Osterhaus AD, et al. Acyclovir-resistant corneal HSV-1 isolates from patients with herpetic keratitis. *J Infect Dis* 2008; 198:659–663.
5. Piret J, Boivin G. Antiviral drug resistance in herpesviruses other than cytomegalovirus. *Rev Med Virol* 2014; 24:186–218.
6. Morfin F, Thouvenot D. Herpes simplex virus resistance to antiviral drugs. *J Clin Virol* 2003; 26:29-37.
7. Swierkosz EM, Hodinka RL, Moore BM, et al. Antiviral susceptibility testing: herpes simplex virus by plaque reduction assay; Approved standard. Wayne, PA: Clinical and Laboratory Standards Institute; 2004; pp. 1–39.
8. Saint-Leger E, Caumes E, Breton G, et al. Clinical and virologic characterization of acyclovir-resistant varicella-zoster viruses isolated from 11 patients with acquired immunodeficiency syndrome. *Clin Infect Dis* 2001; 33:2061–2067.
9. Sauerbrei A. Diagnosis, antiviral therapy, and prophylaxis of varicella-zoster virus infections. *Eur J Clin Microbiol Infect Dis* 2016; 35:723–734.
10. Pramod N, Thyagarajan S, Mohan K, et al. Acyclovir Resistance in Herpes Simplex Virus Isolates from Keratitis Cases: An Analysis from a Developing Country. *Microbiol Immunol.* 2000;44(4):241-247.
11. Abraham AM, Kavitha S, Joseph P, et al. Aciclovir resistance among Indian strains of Herpes simplex virus

- as determined using a dye uptake assay. *Indian J Med Microbiol.* 2007;25(3):260-2.
12. Fife KH, Crumpacker CS, Mertz GJ, Hill EL, Boone GS. Recurrence and resistance patterns of herpes simplex virus following cessation of > or =6 years of chronic suppression with acyclovir. *Acyclovir Study Group. J Infect Dis;* 1994; 169: 1338–41.
  13. Christophers J, Clayton J, Craske J, et al. Survey of resistance of herpes simplex virus to acyclovir in northwest England. *Antimicrob Agents Chemother;* 1998; 42: 868–72.
  14. Charles SJ, Gray JJ. Ocular herpes simplex virus infections: reduced sensitivity to acyclovir in primary disease. *Br J Ophthalmol* 1990; 74: 286–8.
  15. van Velzen M, van de Vijver DA, van Loenen FB, et al. Acyclovir prophylaxis predisposes to antiviral-resistant recurrent herpetic keratitis. *J Infect Dis* 2013; 208:1359–1365.
  16. van Velzen M, van Loenen FB, Meesters RJ, et al. Latent acyclovir-resistant herpes simplex virus type 1 in trigeminal ganglia of immunocompetent individuals. *J Infect Dis* 2012; 205:1539–1543.
  17. Pramod NP, Thyagarajan SP, Mohan KV, et al. Polymerase chain reaction in the diagnosis of herpetic keratitis: experience in a developing country. *Can J Ophthalmol.* 2000;35(3):134-40.
  18. Hlinomazová Z, Loukotová V, Horáčková M, et al. The treatment of HSV1 ocular infections using quantitative real-time PCR results. *Acta Ophthalmol.* 2012;90(5):456-60.
  19. Aliabadi N, Jamalidoust M, Asaei S, et al. Diagnosing of herpes simplex virus infections in suspected patients using real-time PCR. *Jundishapur J Microbiol.* 2015;8(2): e16727.
  20. Ma JX, Wang LN, Zhou RX, et al. Real-time polymerase chain reaction for the diagnosis of necrotizing herpes stromal keratitis. *Int J Ophthalmol.* 2016;9(5):682-6.
  21. Kowalski R, Karenchak L, Dhaliwal D, et al. AmpliVue Is a Practical and Timely Test for the Detection of HSV From Keratitis Specimens. *Eye & Contact Lens: Science & Clinical Practice.* 2017;1. doi:10.1097/icl.0000000000000393.
  22. Hostetler KY. Synthesis and early development of hexadecyloxypropylciclodofovir: an oral antipoxvirus nucleoside phosphonate. *Viruses* 2010; 2:2213–2225.
  23. Wald A, Corey L, Timmler B, et al. Helicase-primase inhibitor pritelivir for HSV-2 infection. *N Engl J Med* 2014; 370:201–210.
  24. Tyring SK, Plunkett S, Scribner AR, et al. Valomaciclovir versus valacyclovir for the treatment of acute herpes zoster in immunocompetent adults: a randomized, double-blind, active-controlled trial. *J Med Virol* 2012; 84:1224–1232.
  25. De SK, Hart JC, Breuer J. Herpes simplex virus and varicella-zoster virus: recent advances in therapy. *Curr Opin Infect Dis* 2015; 28:589–595.

## Recommended reading

- A) Piret J, Boivin G. Antiviral resistance in herpes simplex virus and varicella-zoster virus infections: diagnosis and management. *Curr Opin Infect Dis.* 2016;29(6):654-662.
- B) Herpes Simplex Virus Keratitis: A Treatment Guideline - 2014 [Internet]. American Academy of Ophthalmology. 2014 [cited 24 June 2017]. Available from: <https://www.aao.org/clinical-statement/herpes-simplex-virus-keratitis-treatment-guideline>
- C) Choong K, Walker NJ, Apel AJ, Whitby M. Aciclovir-resistant herpes keratitis. *Clin Experiment Ophthalmol.* 2010;38(3):309-13.
- D) Andrei G, Snoeck R. Herpes simplex virus drug-resistance: new mutations and insights. *Curr Opin Infect Dis.* 2013;26(6):551-60.
- E) Burrel S, Boutolleau D, Azar G, et al. Phenotypic and genotypic characterization of acyclovir-resistant corneal HSV-1 isolates from immunocompetent patients with recurrent herpetic keratitis. *J Clin Virol.* 2013;58(1):321-4.

## Dosage and formulation of Antibiotics / Antifungals / Antiamoebics

**Srujana D, MS**  
INHS Asvini, Mumbai



**Srujana D**

S.No.	Name of antibiotic/ antifungal/ antiamoebics (available dose)	Fortified for topical use: dose & preparation	*Subconjunctival/ #Intracameral: dose & preparation	Intravitreal: dose & preparation
1	Ceftazidime (500 mg)	50 mg/ml (5%) 1. Add 2 ml of distilled water to 500mg 2. Add to 8ml of artificial tears Refrigerate in 4°C Shelf Life: 1week at 4°C and 3 days at room temperature	*Dose : 100 mg/0.5 ml	Intravitreal Ceftazidime : 2.25 mg/0.1 ml 1. Add 2 ml of distilled water to 500mg (250 mg/ml) 2. Take 0.1 ml of solution(25 mg/0.1 ml) 3. Dilute with 0.9 ml of sterile water (25 mg/10 ml) 4. Discard 0.9 ml, use 0.1 ml for injection Can be mixed with steroid preparation in the same syringe. Use dexamethasone in Step 3 if indicated
2	Cephazolin (500 mg)	50 mg/ml (5%) 1. Add 2 ml of distilled water to 500 mg 2. Add to 8 ml of artificial tears Refrigerate in 4°C Shelf Life: 1 week at 4°C and 3 days at room temperature	*Dose : 100 mg/0.5 ml	
2	Vancomycin (500 mg)	50 mg/ml (5%) 1. Add 2 ml of distilled water to 500 mg 2. Add to 8 ml of artificial tears Storage: Refrigerate at 4°C Shelf Life: 28 days at 4°C	*Dose : 25 mg/0.5 ml	Intravitreal Vancomycin : 1 mg/0.1 ml 1. Add 5 ml distilled water to 500 mg of vancomycin powder for injection 2. Mix well (shake)—100 mg/ml 3. Take 0.1 ml of drug solution 4. Dilute with 0.9 ml of sterile water 5. Mix well (moving air bubble up and down) 6. Discard 0.9 ml 7. Use 0.1 ml for injection Caution: Do not mix with other drugs as it gets precipitated
3	Tobramycin	14 mg/ml (1.4%) 1. Add 2 ml (80 mg) of parenteral tobramycin to commercially available tobramycin eye drops 0.3% 5 ml (15 mg/5 ml) Shelf Life: 1 week in refrigerator at 4°C and 4 days in room temperature	*Dose : 20 mg/ 0.5 ml	

S.No.	Name of antibiotic/ antifungal/ antiameobics (available dose)	Fortified for topical use: dose & preparation	*Subconjunctival/ #Intracameral: dose & preparation	Intravitreal: dose & preparation
4	Gentamicin	14 mg/ml (1.4%) Method: Add 2 ml/80 mg of parenteral gentamicin to commercial gentamicin ophthalmic solution 0.3% 5 ml (15 mg/5 ml). Shelf Life: 1 week at 4 degrees C and 4 days in room temperature	*Dose : 20 mg/0.5 ml	
5	Amikacin	2.5% Method: Parenteral Amikacin 250 mg/2 ml is mixed with 8 ml artificial tears. Shelf Life: 7 days at 4 °C		Intravitreal Amikacin: 125 µg/0.1 ml (previously recommended 400 µg was highly retinal toxic) 1. Take 0.1 ml solution (12.5 mg/0.1 ml) of commercially available preparation (250 mg/2 ml) 2. Dilute with 0.9 ml of distilled water (12.5 mg/10 ml) 3. Discard 0.9 ml, take 0.1 ml solution (1.25 mg/0.1 ml) 4. Dilute again with 0.9 ml of distilled water (1.25 mg/10 ml) 5. Use 0.1 ml for injection (125 µg/0.1 ml)
6	Linezolid 200 mg/100 ml (2 mg/ml)	2 mg/ml (0.2%) Parenteral Linezolid available as IV infusion can be directly used		
7	Colistin (1 million IU/75 mg)	0.19% Add to 10ml distilled water – 7.5 mg/ml (0.75%) 1 ml of above solution is then added to 3 ml distilled water		
8	Imipenem (500 mg)– Cilastin (500 mg)	1% Add 10 ml sterile water to create a solution of strength 50 mg/ml. Take 1 ml of this solution and add 4 ml sterile water to make topical Imipenem 1% - 1 mg/ml Storage - In amber coloured bottles Stability – 3 days at 2-8 °C		Intravitreal Imipenem 50– 100 µg/0.1 ml 1. Dilute commercially available preparation with (250 mg) 100 ml of distilled water 2. Take 0.2 ml (0.5 mg) 3. Dilute with 0.3 ml sterile water 4. Inject 0.05 ml
9	Trimethoprim/ sulfamethoxazole 16 mg/mL and 80 mg/mL	Commercial preparation can be used.		
10	Polymyxin B (500,000 units lyophilized powder)	10,000-25,000 units per mL Dissolve 500,000 polymyxin B units in 20 to 50 mL sterile water for injection or sodium chloride injection USP for a 10,000 to 25,000 units per mL concentration. Note: Avoid total systemic and ophthalmic instillation over 25,000 units/kg/day.	Subconjunctival injection of up to 100,000 units/day may be used for the treatment of Pseudomonas aeruginosa keratitis	



S.No.	Name of antibiotic/ antifungal/ antiameobics (available dose)	Fortified for topical use: dose & preparation	*Subconjunctival/ #Intracameral: dose & preparation	Intravitreal: dose & preparation
11	Piperacillin/tazobactam (2 g/250 mg)/vial: (2.25 g)	10% Add 22 ml of 0.9% Sodium Chloride for Injection/ Sterile Water for Injection/ Dextran 6% in Saline/ Dextrose 5% to 2 g/250 mg Piperacillin/tazobactam powder to obtain 10% solution Note: Lactated Ringer's solution is not compatible		Intravitreal 225 µg/0.1 ml
12	Amphotericin B	0.15% 1. Add 10 ml 5% Dextrose to 50 mg of amphotericin B powder. 2. Draw 3 ml of this and add to 7 ml of artificial tears eye drops. Storage: Refrigerate in 4°C. Shelf life: 7 days at 4°C and 4 days in room temperature Phototoxic, to be dispensed in brown bottle	#5 -10 µgm/0.1 ml For 10 µg/0.1 ml : 1. Add 10 ml of 5% Dextrose to create 5 mg/ml. 2. Take 0.2 ml, dilute with 0.8 ml of 5% Dextrose 3. Take 0.1ml, add 0.9ml of 5% Dextrose (0.1 mg/ 1ml) 4. Use 0.1 ml (10 µg/0.1 ml)	Intravitreal amphotericin B (5 µg/0.1 ml) 1. Add 10 ml of 5% Dextrose to commercially available preparation (50 mg) and mix well (5 mg/ml) 2. Take 0.1 ml of drug solution (0.5 mg/0.1 ml) 3. Dilute with 0.9 ml of sterile water (0.5 mg/10 ml) 4. Again take 0.1 ml of solution (50 µg/0.1 ml) and add 0.9 ml distilled water (50 µg /10 ml) 5. Discard 0.9 ml of solution, use 0.1 ml (5 µg/0.1 ml)
13	Voriconazole (200mg)	1% Mix 20 ml ringer lactate to 200 mg voriconazole lyophilized powder. Stability: 30 days at 4°C or room temperature	50 µg/0.1 ml (for intrastromal injection) Method: Take 1 ml from 1% voriconazole solution, add 19 ml ringer lactate to make 0.05 mg/ml (50 µgm/0.1 ml)	Intravitreal Voriconazole 50– 100 µg/0.1 ml 1. Add 19 ml of distilled water to 200 mg voriconazole lyophilized powder (10 mg/ml) 2. Take 0.1 ml solution and add 0.9 ml distilled water and mix well 3. Discard 0.5 ml of solution, take 0.5 ml of sterile water and mix well (air bubble) 4. Discard 0.9 ml of solution, use 0.1 ml for injection (One and a half dilution)
14	Polyhexamethylene biguanide/ Chlorhexidine (20% stock solution)	0.02% 20% stock solution diluted with 0.9% NaCl to obtain 0.02% eye drop solution (0.01 ml of stock solution + 10 ml of 0.9% normal saline=0.02%)		

## References:

- External Disease and Cornea. American Academy of Ophthalmology 1999-2000.
- Prakash G, Sharma N, Goel M, Titiyal JS, Vajpayee RB. Evaluation of intrastromal injection of voriconazole as a therapeutic adjunctive for the management of deep recalcitrant fungal keratitis. Am J Ophthalmol 2008; 146: 56-59.
- Shao Y, Yao Y, Chong Gang P, Tan Y et al. Therapeutic efficacy of intracameral amphotericin B injection for 60 patients with keratomycosis. Int J ophthalmol 2010;3(3):257-260.
- Chew FL, Soong TK, Shin HC, Samsudin A, Visvaraja S.J Ocul Pharmacol Ther. 2010 Apr;26(2):219-22. Topical piperacillin/tazobactam for recalcitrant pseudomonas aeruginosa keratitis.
- Sharma R, Jhanji V, Satpathy G, Sharma N, Khokhar S, Agarwal T. Coinfection with Acanthamoeba and Pseudomonas in contact lens-associated keratitis. Optom Vis Sci. 2013 Feb;90(2):e53-5.
- Dhiman R, Singh A, Tandon R, Vanathi M. Contact lens induced Pseudomonas keratitis following descemet stripping automated endothelial keratoplasty. Cont Lens Anterior Eye. 2015 Oct;38(5):379-81

# Post LASIK Infective Keratitis

**Dr. Pranita Sahay, MD; Dr. Devesh Kumawat, MD;  
Jeewan S Titiyal, MD**

## Corresponding author:

Pranita Sahay, MD, DNB, FICO, FAICO (Refractive)  
Senior Resident, Dr. Rajendra Prasad Centre for Ophthalmic Sciences,  
AIIMS, New Delhi



**Pranita Sahay**

## Introduction

The last decade has seen a rising trend in the number of patients opting for laser vision correction (LVC) for refractive error. The various options for laser vision correction include photorefractive keratectomy (PRK), laser subepithelial keratomileusis (LASEK), laser in situ keratomileusis (LASIK) and Small Incision Lenticule Extraction (SMILE). LASIK still remains one the most commonly performed LVC surgery. Complications of LASIK include dry eye, glare, halo, under-correction and over-correction.(1) Post LASIK microbial keratitis is one of the most dreaded sight threatening complication that can result in permanent loss of patients best corrected visual acuity (BCVA).(1) The incidence of post LASIK microbial keratitis reported in literature vary from 0%-1.5%.(2)

## Risk Factors

As prevention is better than cure, following risk factors should be kept in mind while assessing every case and avoided as far as possible.(3)

### 1. Pre-operative

All cases planned for any laser refractive surgery should be screened for the following risk factors

- a. Dry eye disease
- b. Meibomian gland dysfunction
- c. Blepharitis
- d. Immunocompromised status (HIV positive)

### 2. Intra-operative

- a. Breach in OT sterilization or surgical asepsis
- b. Use of same microkeratome blade for multiple cases
- c. Corneal epithelial defect
- d. Presence of interface debris

### 3. Post-operative

- a. Use of a bandage contact lens (BCL)
- b. Splashing of tap water in the eyes
- c. Post traumatic flap dislocation
- d. Poor compliance with medications

## Etiology

Post LASIK infective keratitis can be classified into early and late based on its onset from surgery. The microorganisms implicated are different for these two conditions.(2)

1. Early onset infectious keratitis: It is defined as infective keratitis occurring within 2 weeks of surgery. The microorganisms implicated in early onset infective keratitis are primarily gram-positive bacteria like *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus viridians* and *Streptococcus pneumoniae*. Gram negative organisms like *Pseudomonas aeruginosa* are less commonly associated.(2)
2. Late onset infectious keratitis: It is defined as infective keratitis occurring between 2 weeks to 3 months after surgery. The microorganisms most commonly implicated in late-onset infective keratitis are fungi, atypical mycobacteria and *Nocardia*.(2,4) The atypical mycobacteria are present in the environment, soil and body surface, and can readily cause infection as they are highly resistant to chemical disinfection. (5,6)

## Clinical Features

### Symptoms

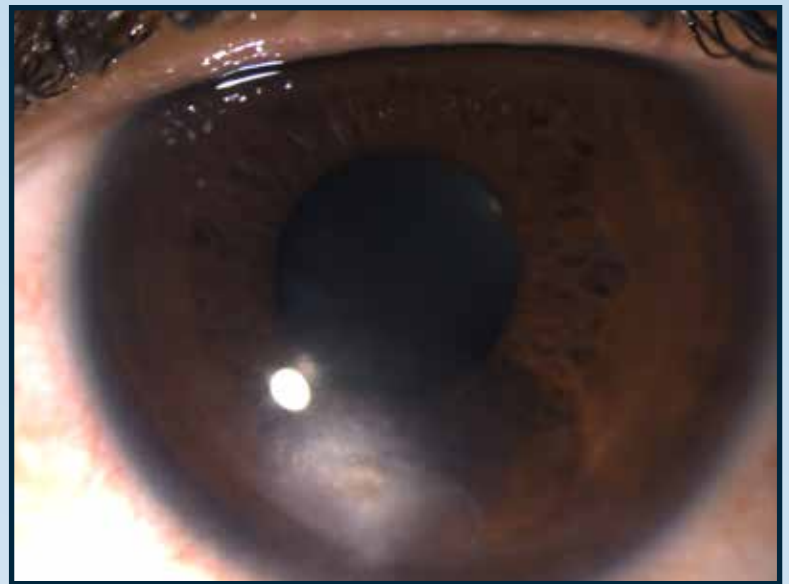
The onset of symptoms usually occurs after 2-3 days of surgery. Patient presents with complaints of pain, redness, watering, photophobia, diminution of vision, foreign body sensation and ocular irritation. (2) The symptoms of pain may not be as severe as seen in routine cases of infective keratitis due to severing of the corneal nerves during LASIK.

### Signs

On examination, there are corneal infiltrates which is not only limited to the interface but may also involve the flap as well as the deep stromal tissue.(Fig-1, Fig-2) There is inflammatory reaction in the anterior chamber as well.(5) In severe cases, there may be associated flap melting, which heals with corneal scarring resulting in irregular astigmatism. (7)



**Figure 1.** Slit lamp photograph of right eye showing deep stromal infiltrates with total cataract post LASIK



**Figure 2.** Slit lamp photograph of left eye showing resolving keratitis with stromal infiltrate and surrounding scar post LASIK

The corneal infiltrate in case of microbial keratitis with gram positive organisms are usually located at the flap edge and have a well defined margin while that due to atypical Mycobacteria and fungi are located at the interface and have an indistinct and feathery margin.(8)

### Differential Diagnosis

Other conditions which may be considered as differential diagnosis are(9)

1. Diffuse lamellar keratitis
2. Interface debris
3. Sterile peripheral infiltrates
4. Epithelial in-growth

The most important differential diagnosis for a case of post LASIK infective keratitis is diffuse lamellar keratitis. The differentiating features have been highlighted in Table 1.

**Table 1.**

Characteristics	Infective Keratitis	Diffuse Lamellar Keratitis
Etiology	Staph. aureus, Staph. epidermidis, Strept. Pneumonia, atypical mycobacteria, Fungi, Nocardia	Inflammatory reaction
Onset	After 2-3 days of surgery	Within first 24 hours
Symptoms	Moderate to severe pain and photophobia	Mild pain and photophobia
Conjunctival congestion	Moderate to severe	Mild
Lid oedema	Moderate to severe	Mild
Location of infiltrate	Interface, may involve the flap and deep stroma	Confined to interface, begins in the flap periphery
Appearance	Focal area of infiltration surrounded by diffuse inflammation	Diffuse interface inflammation; called sands of Sahara
Anterior chamber reaction	Present	Absent

## Management

The management of Post LASIK keratitis has been comprehensively described by Donnenfeld et al. (Figure 3) (3) In most of the cases there are no superficial corneal infiltrate that can be scraped for sampling. Therefore, it is recommended to lift the flap and scrape the bed for obtaining samples both for smear and culture sensitivity examination.(3) The culture media includes blood agar and chocolate agar for bacteria, Sabouraud's dextrose agar for fungus, and Thioglycolate broth for anaerobic organisms. In case of late onset infection, atypical mycobacteria needs to be ruled out and therefore sample is sent in Lowenstein-Jensen or Middlebrook 7H9 agar in addition to the other culture media. The sample is also subjected to smear examination with Gram stain, Gomori-methenamine silver stain, and Ziehl-Neelsen stain to rule out unusual pathogens such as *Nocardia*, atypical mycobacteria, and fungi. In cases where all reports are negative, corneal biopsy or polymerase chain reaction is done. In addition, confocal microscopy may be used as an adjunctive non-invasive imaging tool for detection of fungal hyphae, *Acanthamoeba* trophozoites and cysts.

The treatment of all cases of post LASIK infective keratitis consists of flap lift with scraping of bed for procuring samples for microbiological evaluation. This is followed by irrigation of the flap interface with an antibiotic solution, which consists of fortified vancomycin 5% (50 mg/mL) for early onset cases and fortified amikacin 3.5% (35 mg/mL) for late onset cases of infective keratitis. (3)

Empirically, all cases of early onset infection are started on topical fourth-generation fluoroquinolone (gatifloxacin 0.3% or moxifloxacin 0.5%) along with fortified cefazolin 5% (50 mg/mL) on hourly regimen round the clock for first 48 hours and then 2 hourly regimen until there is clinical response. In case the patient works in a hospital environment and has risk of exposure to methicillin-resistant *Staphylococcus aureus* (MRSA), topical vancomycin 5% (50 mg/mL) is started instead of cefazolin as it is more effective against MRSA. In addition, oral doxycycline, which has anti-collagenase effect, is given 100 mg BD to prevent further collagenolysis. In case of late-onset keratitis, amikacin 3.5% (35 mg/mL) along with a fourth-generation fluoroquinolone (gatifloxacin 0.3% or moxifloxacin 0.5%) is preferred on hourly regimen. Topical clarithromycin 1% (10mg/mL) and oral clarithromycin 500 mg BD is also effective

and are used in addition topical amikacin 3.5%. (2,6,8) Natamycin 5% (50mg/mL) for filamentous fungi and amphotericin B 0.15% (1.5mg/mL) for yeast infection and topical Voriconazole 1% (10mg/mL) with oral Voriconazole 200 mg BD is effective against both filamentous and yeast infections.(4,10) Topical corticosteroids are discontinued in all these cases. The treatment is further modified based on the culture and sensitivity reports.

In case of progressive keratitis, not responding to medical treatment, partial or total amputation of the flap, based on the location and size of infiltrate is done to allow better penetration of the drug.(11) The healing of infective keratitis results in scar formation and irregular astigmatism. Visual rehabilitation of these cases is later done with either rigid gas permeable contact lens or lamellar/penetrating keratoplasty.

## Prophylaxis

The following standard precautions can reduce the risk of post LASIK infective keratitis.(3)

1. Preoperative: Treatment of meibomian gland disease
2. Intraoperative
  - a) Proper sterilization of instruments
  - b) Separate instruments for both eyes when performing bilateral surgery
  - c) Use of sterile drapes, gowns, gloves, and masks by the treating physician and assisting technician
  - d) Povidone-iodine solution (Betadine 10%) for lid preparation
  - e) Application of drape to remove the lashes from the surgical field
  - f) All fluids applied to the eye before, during, and after LASIK should be sterile
3. Post-operative
  - a) Topical antibiotic (4<sup>th</sup> generation fluoroquinolone)
  - b) Use of eye shields in the early post operative period to avoid trauma
  - c) Avoid rubbing of eyes
  - d) Avoid direct application of tap water in eyes, swimming and gardening
  - e) Avoid eye make-up
  - f) Frequent lubrication in subjects with dry eye

## Conclusion

PostLASIKinfectivekeratitisisadreadedcomplication of refractive surgery. Proper pre-operative, intra-operative and post-operative precautions can avert this sight threatening complication. In addition, an early diagnosis with timely initiation of appropriate treatment (medical/surgical) is necessary for best outcome.

## References

1. Melki SA, Azar DT. LASIK Complications: Etiology, Management, and Prevention. *Surv Ophthalmol*. 2001 Sep;46(2):95–116.
2. Chang MA, Jain S, Azar DT. Infections following laser in situ keratomileusis: an integration of the published literature. *Surv Ophthalmol*. 2004 Jun;49(3):269–80.
3. Donnenfeld ED, Kim T, Holland EJ, Azar DT, Palmon FR, Rubenstein JB, et al. ASCRS White Paper: Management of infectious keratitis following laser in situ keratomileusis. *J Cataract Refract Surg*. 2005 Oct;31(10):2008–11.
4. Sahay P, Maharana PK, Sharma N, Titiyal JS. Post laser-assisted in situ keratomileusis keratomycotic malignant glaucoma. *BMJ Case Rep*. 2017 Oct 13;2017.
5. Karp CL, Tuli SS, Yoo SH, Vroman DT, Alfonso EC, Huang AH, et al. Infectious keratitis after LASIK. *Ophthalmology*. 2003 Mar;110(3):503–10.
6. Nascimento H, Viana-Niero C, Nogueira CL, Martins Bispo PJ, Pinto F, de Paula Pereira Uzam C, et al. Identification of the Infection Source of an Outbreak of Mycobacterium Chelonae Keratitis After Laser in Situ Keratomileusis. *Cornea*. 2018 Jan;37(1):116–22.
7. Randleman JB, Shah RD. LASIK Interface Complications: Etiology, Management, & Outcomes. *J Refract Surg Thorofare NJ* 1995. 2012 Aug;28(8):575–86.
8. Fulcher SFA, Fader RC, Rosa RH, Holmes GP. Delayed-onset mycobacterial keratitis after LASIK. *Cornea*. 2002 Aug;21(6):546–54.
9. Sanz JLA y, Azar DT. Management of Complications in Refractive Surgery. Springer Science & Business Media; 2008. 378 p.
10. Mittal V, Jain R, Mittal R, Sangwan VS. Post-laser in situ keratomileusis interface fungal keratitis. *Cornea*. 2014 Oct;33(10):1022–30.
11. Kamiya K, Kasahara M, Shimizu K. A case of intractable infectious keratitis and subsequent flap necrosis after laser in situ keratomileusis. *Clin Ophthalmol Auckl NZ*. 2009;3:523–5.

# Types of Riboflavin & Methods of Corneal Crosslinking

**Deepali Singhal, MD; Pranita Sahay, MD;  
Prafulla K. Maharana, MD**

**Corresponding author:**

Prafulla K. Maharana

Assistant Professor, Department of Ophthalmology,

Room- S5, 1st floor, Dr. Rajendra Prasad Centre for Ophthalmic Sciences,

All India Institute of Medical Sciences, New Delhi-110029, India

Email ID: drpraful13@gmail.com



**Deepali Singhal**

## Introduction

Keratoconus (KC) is an asymmetric, progressive corneal ectasia that can lead to significant visual impairment due to associated myopia and irregular astigmatism. The gold standard for the management of progressive KC has been corneal collagen crosslinking (CXL), using ultraviolet-A (UVA) radiation and the photosensitizer riboflavin. This procedure has the potential to stop the progression of keratoconus, avoiding the need for penetrating keratoplasty.(1)

Keratoconus progression varies with age with most rapid progression is seen in puberty, which continues throughout adolescence, and stabilizes at around third or fourth decade of life. This stabilization has been explained by the age-related increase in corneal stiffness which is due to the glycation induced cross-linking between or within stromal collagen lamellae, as a result of the accumulation of non-enzymatic glycation end products over time.(1)

## Corneal collagen crosslinking (CXL)

CXL was introduced in 1995, as a technique to increase the mechanical stiffness of connective tissue. (2,3) In this technique 0.1% aqueous solution of riboflavin 5 phosphate (vitamin B2) acts as a photomediator and absorbs the UV A radiation (370 nm) to generate free radicals thereby, inducing the formation of additional intra-molecular collagen crosslinks. This molecule also provides adequate shielding of the deeper tissues such as the endothelium, lens and macula. This technique has been shown to arrest progression of keratoconus due to increase in biomechanical strength of the human cornea by approximately 300%.(3)

The riboflavin solution is diluted in dextran 20%, a carrier to make it iso-osmolar to the corneal stroma in order to avoid any stromal swelling. The corneal epithelium acts as a barrier against the penetration of this macromolecule (riboflavin) which necessitates the debridement of epithelium prior to instillation of riboflavin.(4)

## Mechanism

When activated with UVA light, riboflavin is converted into excited singlet and triplet states, which undergo a series of chemical reactions subsequently, by two mechanisms. A mechanism favored at low oxygen concentration (Type I), produces radical riboflavin, while a mechanism favored at higher oxygen concentrations (Type II), produces singlet molecular oxygen. The radical riboflavin and reactive oxygen species produced by the Type I & II photochemical mechanisms are thought to interact with corneal proteins (such as tyrosine and tryptophan) to create chemical bonds that result in corneal cross-linking. Therefore, the presence of riboflavin in the cornea both protects the internal structures of the eye from UVA transmittance and induces the cross-linking effect.(5)

## The Dresden (standard) protocol

This standard protocol uses iso-osmolar 0.1% riboflavin solution, which is generated by diluting vitamin B2-riboflavin-5-phosphate 0.5% with

dextran T500 20% (402.7 mOsmol/L). After a 9 mm diameter debridement of the corneal epithelium, iso-osmolar riboflavin solution is applied on the cornea every 3 minutes for 30 minutes. Following this, ultrasound pachymetry (five repetitive measurements) should be performed at the thinnest point to ensure a minimal stromal thickness of 400  $\mu\text{m}$ .(3,6) Successful penetration of riboflavin is ensured by visualization of riboflavin in the anterior chamber on slit-lamp biomicroscopy. The eye is then irradiated for 30 minutes with UV-A at an intensity of 3 mW/cm<sup>2</sup>, corresponding to a surface dose of 5.4 J/cm<sup>2</sup> at a working distance of 5 cm (UV-X; IROC Medical, Zurich, Switzerland; distributed by Peschke Meditrade GmbH, Nuremberg, Germany) to induce corneal crosslinks to a depth of around 310  $\mu\text{m}$ . An isotonic 0.1% riboflavin solution and a topical anesthetic (oxybuprocaine 0.4% or 0.5% proparacaine) are administered every 5 minutes to saturate the cornea with riboflavin.

This protocol has been reported to be efficacious in slowing or halting the progression of ectasia in patients with keratoconus, with a significant improvement in maximum keratometry (Kmax) or cone apex power in CXL-treated eyes relative to controls at 12 months, postoperatively.(7,8) A commonly used riboflavin is the Peschke® D standard riboflavin (0.1%) solution with 20% dextran 500.

## Modifications for thin corneas

According to the current inclusion criteria a minimal stromal thickness (after debridement of the corneal epithelium) of 400  $\mu\text{m}$  is required for performing CXL in order to avoid endothelial damage. However, to treat the patients with thin corneas various modifications have been introduced to induce stromal swelling and increase the stromal thickness prior to CXL.

### Hypo-osmolar Riboflavin (0.1%)

Hypo-osmolar 0.1% riboflavin solution is formed by diluting vitamin B2-riboflavin-5-phosphate 0.5% with physiologic salt solution (sodium chloride 0.9% solution; 310 mOsmol/L; B. Braun Medical AG, Sempach, Switzerland) instead of dextran. The procedure performed is similar as described above that is after removal of the corneal epithelium and 30 minutes of instillation of iso-osmolar riboflavin solution, the corneal stromal thickness is measured using ultrasound pachymetry. In cases where the thickness is less than than 400  $\mu\text{m}$ , hypo-

osmolar riboflavin is applied every 20 seconds for 5 additional minutes, and the corneal thickness is again measured following which the administration of riboflavin is continued till the thickness reaches 400  $\mu\text{m}$ .

The absolute increase in the corneal thickness reported in a case series ranged from 36 to 110  $\mu\text{m}$ , where the thinnest cornea was 323  $\mu\text{m}$  after removal of the epithelium. The results of this technique are noted to be similar to those of the standard protocol (ie, an isoosmolar solution).(9)

## Different types of riboflavin commonly used are:

- Peschke® D: Standard riboflavin solution for epi-off procedure containing 0.1 % Riboflavin (Vitamin B2) with 20% Dextran (Dresden original)
- Peschke® M: Standard riboflavin solution without dextran for epi-off procedure containing 0.1 % Riboflavin (Vitamin B2) with 1.1 % HPMC.
- Peschke® H: a hypotonic riboflavin solution for inducing corneal swelling containing 0.1 % Riboflavin (Vitamin B2)

## Transepithelial Corneal Collagen Cross-linking

Riboflavin is known not to readily penetrate the intact epithelium, this has led to the development of different techniques to increase the absorption of riboflavin into the stroma, including the use of eye drops containing preservatives, such as benzalkonium chloride (BAK) preoperatively. (10) The efficacy of transepithelial CXL has been reported to be only one fifth of the conventional CXL with complete epithelial removal. (11) The main advantages of this technique include improved patient comfort and decreased risk of infection associated with epithelial removal.

Riboflavin with BAK has been shown to effectively penetrate the intact epithelium. BAK acts as a tensioactive substance and changes the surface tension value, facilitating the penetration of other substances through biological membranes. It has been shown in experimental studies that with this technique, the epithelial surface retains its curvature due to increased stiffness with more compact arrangement and straightening of the stromal fibres. This effect was noticed in the upper 50% of the stroma. Thus, tensioactive CXL is a less invasive

procedure with fewer complications and increased patient comfort. (12)

One of the examples of riboflavin used for this technique is Peschke® TE which is recommended to be instilled for a duration of 20 minutes (1 drop every 2 minutes = 10 drops) and contains 0.25 % Riboflavin (Vitamin B2) with 1.2 % HPMC and 0.01 % Benzalkoniumchloride.

### **Riboflavin with ethylenediaminetetraacetic acid (EDTA)**

Filippello et al has shown the demarcation line to be more superficial (100  $\mu\text{m}$ ) as compared to that in conventional CXL (320 to 340  $\mu\text{m}$ ) using 0.1% aqueous riboflavin involving trometamol and sodium EDTA to break down epithelial intercellular junctions. (13) This may suggest a more superficial effect of transepithelial CXL.

## **Other modifications**

### **Customized pachymetry-guided epithelial debridement**

This technique includes cone-on CXL that is preservation of the epithelium over the cone (thinnest area of cornea) or area of maximal topographical steepening. [14] However, this technique has been shown to have a limited penetration of riboflavin with a demarcation line reported to be at 150  $\mu\text{m}$ .(15)

### **Contact lens assisted CXL (CLCXL)**

Riboflavin-soaked bandage contact lens has been used effectively in thin corneas for CXL and is reported to augment the corneal thickness by 100  $\mu\text{m}$  approximately. [16] However, the major limitations include inability to customize the contact lens thickness and intraoperative buckling. Moreover, the absorption properties of contact lenses may be different from that of the corneal stroma. Long-term results of this procedure are yet not available.

### **Refractive lenticule assisted CXL**

Augmentation of stromal thickness for CXL in thin corneas has also been achieved using refractive lenticules obtained from patients undergoing small incision lenticule extraction (SMILE) for myopic correction.[17] This technique is believed to be the most physiological method to increase the stromal thickness. Moreover, the rough host stromal surface allows the lenticule to spread easily and buckling is avoided.

## **Hydroxypropyl methylcellulose (HPMC)**

### **Riboflavin**

The use of hydroxypropyl methylcellulose riboflavin has been shown to prevent corneal dehydration induced by dextran and is more suitable for thin corneas. [18] Riboflavin solution with 20% dextran leads to approximately 30% corneal thinning, whereas a hydroxypropyl methylcellulose (HPMC)–containing solution resulted in less than 10% corneal thinning and a hypotonic saline solution resulted in 10% corneal swelling. (19)

### **Adapted fluence**

This technique is evolving as the latest technique for thin corneas and is based on the principle of customizing UV-A irradiance according to the stromal thickness. The UV-A fluence delivered in all the previously described techniques is constant (5.4 J/cm<sup>2</sup>) whereas, this technique involves delivering of customized energy by altering the irradiation time (UVA irradiation of 3 mW/cm<sup>2</sup> for a customized irradiation time).(20) This also eliminates the need for varying riboflavin concentrations and overcomes the limitations associated with other techniques.

### **Iontophoresis (I-CXL)**

Iontophoresis is a noninvasive technique that involves application of a mild electric current and allows effective penetration of transepithelial riboflavin. Since, riboflavin is a negatively charged, water-soluble molecule with a relatively low molecular weight, it is suitable for iontophoresis.(21,22) Initial studies have demonstrated stabilization of the disease process following iontophoresis-assisted CXL (I-CXL).(23) However, the keratometric regression was lower as compared to conventional epi-off cross-linking.(24) Also the depth of penetration and concentration achieved is noted to be deeper and greater as compared to transepithelial CXL but still less than epi off conventional method.(25) Thus, I-CXL has the potential to become an alternative treatment for keratoconus while reducing the treatment time, postoperative patient discomfort as well as risk of infection.

### **Accelerated CXL**

Accelerated CXL aims at reducing the duration of the procedure and improving the patient comfort. These protocols are based on the fundamental law of photochemistry, called as the Bunsen-Roscoe Law of Reciprocity. (26,27) This law states



that photochemical biological effect of ultraviolet is proportional to the total energy dose delivered, regardless of the applied irradiance and time. This law holds true within the range of irradiances of currently available UVA delivery devices. The energy dose delivered by a UVA source (joules per square centimeter) is the product of the irradiance of the source (watts per square centimeter) and the delivery time (seconds).

However, several studies have evaluated the safety and efficacy of accelerated protocols in the range of 7 to 45 mW/cm<sup>2</sup> irradiation with a similar efficacy and visual outcome without any significant endothelial cell loss.(26,28-30) Thus, the Bunsen and Roscoe reciprocity law is valid only for illumination intensity up to 45 mW/cm<sup>2</sup> and an irradiance time >2 min. However, the demarcation line is reported to be more pronounced and more anteriorly located than the standard protocol.(30-32) Thus, a shallow demarcation line in accelerated CXL also indicates reduced risk of endothelial cytotoxicity. (33)

The major limitation reported with this protocol is the reduced corneal stiffening effect with increasing intensity of UV-A radiation at more than 18 mW/cm<sup>2</sup>. This is mainly due to the rapid oxygen consumption and failure of Type I photochemical reaction at an intensity of more than 10 mW/cm<sup>2</sup> thereby leading to reduced efficacy and higher failure rates.(34)

### **Pulsed illumination**

Pulsed illumination during CXL is based on the concept that illumination with UVA irradiation causes a rapid depletion of oxygen in cornea saturated with riboflavin, while turning the UVA lamp off leads to rapid replenishment of the oxygen to its original level by diffusion from the tissues.(12) This further acts to create additional cross-linking interactions. (35,36) This technique using 0.1% riboflavin with HPMC (VibeX Rapid; Avedro) with a UVA dose of 7.2 J/cm<sup>2</sup> with a pulse interval of 1 seconds has been reported to have a deeper demarcation line (200 μm) as compared to 160 μm in continuous group. No endothelial cell loss was observed in either group. (37)

However, further studies are required to determine the ideal pulsing duration and approach.

### **Customized cross linking / Photorefractive intrastromal CXL (PiXL)**

Various authors have studied the factors predicting the outcome of CXL. The factors independently predictive of improvement after CXL were CDVA of 20/40 or worse or Kmax of 55.0 D or greater. (38, 39) There was no role of corneal thickness, preoperative corneal haze, and cone location in predicting the outcome of CXL. However, a more eccentric cone has also been shown to be associated with a higher keratometry at 1year follow-up. (40)

This technique involves delivering of customized UVA treatment patterns localized on specific corneal zones. This pattern is generated using a three-dimensional finite element analysis (FEA) model. (41) The KXL II; Avedro device incorporating iris tracking and programmable UVA patterns has recently been granted CE Mark in Europe and is being used to study customized CXL (referred to as photorefractive intrastromal CXL [PiXL]). The UVA LED source illuminates a digital micromirror device (DMD). This technique has shown a significant improvement in visual acuity in keratoconus as well as in patients with hyperopia.(42,43)

### **Laser in situ keratomileusis Xtra (LASIK Xtra)**

This technique is based on the concept of compensating the reduced biomechanical strength of the cornea in LASIK. However, the major concern is the flattening effect resulting in hyperopic shift as well as the post-CXL stromal haze, which can contribute to deterioration in visual quality. [44]

The LASIK Xtra procedure involves application of a higher concentration (0.25%) riboflavin on the stromal bed subsequent to excimer laser ablation for a duration of 90 s followed by washing of interface and repositioning of the flap. UV-A irradiance is then delivered as a homogenous beam of 30 mW/cm<sup>2</sup> for 90 s (2.7J/cm<sup>2</sup>). This delivers around half the energy used in conventional cross- linking in the Dresden protocol, which helps to restore or improve corneal strength without inducing a refractive change.

Various studies have shown a greater refractive accuracy and stability with a lower incidence of regression in hyperopes with this procedure. (45-47)

One of the examples of riboflavin used for this technique is Peschke® L which contains > 0.23 % Riboflavin (Vitamin B2).

## Conclusion

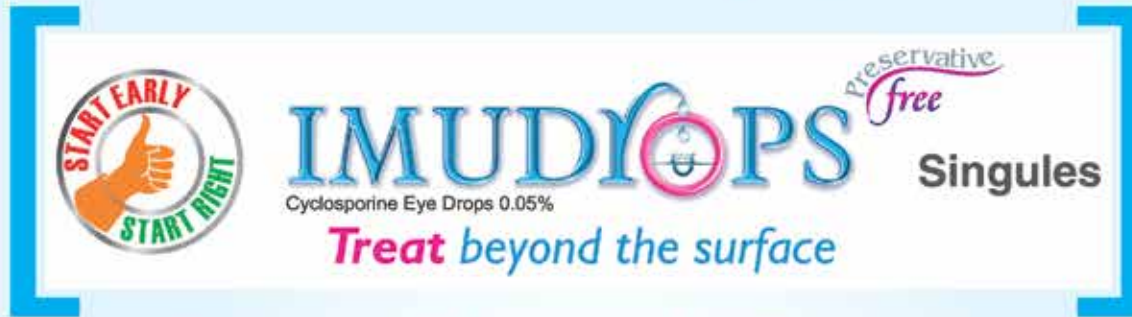
CXL with riboflavin and UVA irradiation is a minimal invasive technique that modifies corneal stroma and increases the biomechanical stability. It is still an evolving technology and has the potential to maximize the visual rehabilitation with various upcoming modifications in the technique.

## References

1. Mas Tur V, MacGregor C, Jayaswal R, O'Brart D, Maycock N. A review of keratoconus: Diagnosis, pathophysiology, and genetics. *Surv Ophthalmol*. 2017 Nov - Dec;62(6):770-783.
2. Spoerl E, Huhle M, Seiler T. Induction of cross-links in corneal tissue. *Exp Eye Res* 1998;66:97-103.
3. Wollensak G, Spoerl E. Collagen crosslinking of human and porcine sclera. *J Cataract Refract Surg*. 2004;30(3):689-695.
4. Prausnitz MR, Noonan JS. Permeability of cornea, sclera, and conjunctiva: a literature analysis for drug delivery to the eye. *J Pharm Sci*. 1998;87(12):1479-1488.
5. Kamaev P, Friedman MD, Sherr E, et al. Photochemical kinetics of corneal cross-linking with riboflavin. *Invest Ophthalmol Vis Sci* 2012;53:2360-2367.
6. Spoerl E, Mrochen M, Sliney D et al. Safety of UVA-riboflavin cross-linking of the cornea. *Cornea*. 2007;26(4):385-389.
7. Wittig-Silva C, Chan E, Islam FMA, et al. A randomized, controlled trial of corneal collagen cross-linking in progressive keratoconus: Three-year results. *Ophthalmology* 2014;121:812-821.
8. O'Brart DP, Kwong TQ, Patel P, et al. Long-term follow-up of riboflavin/ultraviolet A (370 nm) corneal collagen cross-linking to halt the progression of keratoconus. *Br J Ophthalmol* 2013;97:433-437.
9. Hafezi F, Mrochen M, Iseli HP, Seiler T. Collagen Cross-Linking with UVA and hypoosmolar Riboflavin Solution in thin corneas. *J Cataract Refract Surg*.
10. Hayes S, O'Brart DP, Lamdin LS, et al. Effect of complete epithelial debridement before riboflavin-ultraviolet-A corneal collagen crosslinking therapy. *J Cataract Refract Surg*. 2008;34:657Y661.
11. Wollensak G, Iomdina E. Biomechanical and histological changes after corneal crosslinking with and without epithelial debridement. *J Cataract Refract Surg*. 2009;35:540Y546.
12. El Shawaf HI. TCCXL: Transepithelial corneal cross-linking—Italian results. Paper presented at the IV International Congress of CXL; December 5-6, 2008; Dresden, Germany.
13. Filippello M, Stagni E, O'Brart D. Transepithelial corneal collagen crosslinking: bilateral study. *J Cataract Refract Surg*. 2012;38:283Y291.
14. Kymionis GD, Diakonis VF, Coskunseven E, Jankov M, Yoo SH, Pallikaris IG, et al. Customized pachymetric guided epithelial debridement for corneal collagen cross linking. *BMC Ophthalmol*. 2009;9:10. [PMCID: PMC2744909] [PubMed: 19715585]
15. Kaya V, Utine CA, Yilmaz OF. Efficacy of corneal collagen cross-linking using a custom epithelial debridement technique in thin corneas: A confocal microscopy study. *J Refract Surg*. 2011;27:444-50. [PubMed: 21162472]
16. Kumar DA, Agarwal A, Basu S, Sinha P, Agarwal A, et al. Contact lens-assisted collagen cross-linking (CACXL): A new technique for cross-linking thin corneas. *J Refract Surg*. 2014;30:366-72. [PubMed: 24972403]
17. Sachdev MS, Gupta D, Sachdev G, Sachdev R. Tailored stromal expansion with a refractive lenticule for crosslinking the ultrathin cornea. *J Cataract Refract Surg*. 2015;41:918-23. [PubMed: 25953470]
18. . Mazzota C, Baiocchi S, Caparossi T, Craquilli S, Paradiso AL, Caparossi A. Riboflavin 0.1% (vibex) for the treatment of keratoconus. *Expert Opin Orphan Drugs*. 2013;1:235-40.
19. Sherr EA, Kamaev P, Rood-Ojalvo S, et al. Impact of riboflavin formulations on corneal hydration. *Invest Ophthalmol Vis Sci* 2013;54. E-Abstract. 5274.
20. Hafezi F. New Nomogram for Extremely thin KC Corneas: Clinical. CXL Experts Meet. Zurich, Switzerland; 2.3 December. 2016
21. Bikbova G, Bikbov M. Transepithelial corneal collagen cross-linking by iontophoresis of riboflavin. *Acta Ophthalmol*. 2014;92:e30-4. [PubMed: 23848196]
22. Vinciguerra P, Rechichi M, Rosetta P. High fluence iontophoretic corneal collagen crosslinking;In vivo OCT imaging of riboflavin penetration. *J Refract Surg*. 2013;29:376-7. [PubMed: 23739828]
23. Vinciguerra P, Randleman JB, Romano V, Legrottaglie EF, Rosetta P, Camesasca FI, et al. Transepithelial iontophoresis corneal collagen cross-linking for progressive keratoconus: Initial clinical outcomes. *J Refract Surg*. 2014;30:746-53. [PubMed: 25375847]
24. Bikbova G, Bikbov M. Standard corneal collagen crosslinking versus transepithelial iontophoresis- assisted corneal crosslinking, 24 months follow-up: Randomized control trial. *Acta Ophthalmol*. 2016;94:e600-6. [PMCID: PMC5111766] [PubMed: 27040458]
25. Mastropasqua L, Nubile M, Calienno R, Mattei PA, Pedrotti E, Salgari N, et al. Corneal cross-linking: Intrastromal riboflavin concentration in iontophoresis-assisted imbibition versus traditional and transepithelial techniques. *Am J Ophthalmol*. 2014;157:623-30. [PubMed: 24321474]
26. Bunsen R, Roscoe HE. Photochemische Untersuchungen. *Poggendorff's Annalen* 1855;96:373-394.
27. Gatziofufas Z, Richoz O, Brugnoli E, et al. Safety profile of high-fluence corneal collagen cross-linking for progressive keratoconus: Preliminary results from a prospective cohort study. *J Refract Surg* 2013;29: 846-848.
28. Kanellopoulos AJ. Long term results of a prospective randomized bilateral eye comparison trial of higher

- fluence, shorter duration ultraviolet A radiation, and riboflavin collagen cross linking for progressive keratoconus. *Clin Ophthalmol* 2012;6:97–101.
29. Mita M, Waring GO, Tomita M. High-irradiance accelerated collagen cross-linking for the treatment of keratoconus: Six-month results. *J Cataract Refract Surg* 2014;40:1032–1040.
  30. Tomita M, Mita M, Huseynova T. Accelerated versus conventional corneal collagen crosslinking. *J Cataract Refract Surg* 2014;40:1013–1020.
  31. Kanellopoulos AJ, Asimellis G. Introduction of quantitative and qualitative corneal optical coherence tomography findings induced by collagen cross-linking for keratoconus: A novel effect measurement benchmark. *Clin Ophthalmol* 2013;7: 329–335.
  32. Touboul D, Efron N, Smadja D, et al. Corneal confocal microscopy following conventional, transepithelial, and accelerated corneal collagen cross-linking procedures for keratoconus. *J Refract Surg* 2012;28: 769–776.
  33. Kymionis GD, Tsoulnaras KI, Grentzelos MA, et al. Corneal stroma demarcation line after standard and high-intensity collagen crosslinking determined with anterior segment optical coherence tomography. *J Cataract Refract Surg* 2014;40:736–740.
  34. Mrochen M. Current status of accelerated corneal cross-linking. *Indian J Ophthalmol* 2013;61:428.
  35. Kamaev P, Friedman MD, Sherr E, et al. Photochemical kinetics of corneal cross-linking with riboflavin. *Invest Ophthalmol Vis Sci* 2012;53:2360–2367.
  36. Herekar SV. Method for equi-dosed time fractionated pulsed UVA irradiation of collagen/riboflavin mixtures for ocular structural augmentation. US Patent 20090149923 A1. June 11, 2009.
  37. Mazzotta C, Traversi C, Caragiuli S, et al. Pulsed vs continuous light accelerated corneal collagen crosslinking: In vivo qualitative investigation by confocal microscopy and corneal OCT. *Eye (Lond)* [published online ahead of print July 25, 2014]. doi:10.1038/eye.2014.163.
  38. Koller T, Pajic B, Vinciguerra P, et al. Flattening of the cornea after collagen crosslinking for keratoconus. *J Cataract Refract Surg* 2011;37:1488–1492.
  39. Greenstein SA, Hersh PS. Characteristics influencing outcomes of corneal collagen crosslinking for keratoconus and ectasia: Implications for patient selection. *J Cataract Refract Surg* 2013;39:1133–1140.
  40. Wisse RPL, Godefrooij Da, Soeters N, et al. A multivariate analysis and statistical model for predicting visual acuity and keratometry one year after cross-linking for keratoconus. *Am J Ophthalmol* 2014;157:519–525.e2.
  41. Roy AS, Dupps WJ. Patient-specific computational modeling of keratoconus progression and differential responses to collagen cross-linking. *Invest Ophthalmol Vis Sci* 2011;52:9174–9187.
  42. Kanellopoulos AJ, Dupps WJ, Seven I, et al. Toric topographically customized transepithelial, pulsed, very high-fluence, higher energy and higher riboflavin concentration collagen cross-linking in keratoconus. *Case Rep Ophthalmol* 2014;5:172–180.
  43. Kanellopoulos AJ, Asimellis G. Hyperopic correction: Clinical validation with epithelium-on and epithelial-off protocols, using variable fluence and topographically customized collagen cross-linking. *Clin Ophthalmol*. 2014;8:2425–33. [PMCID: PMC4259508] [PubMed: 25506204]
  44. Sorkin N, Varssano D. Corneal collagen crosslinking: A systematic review. *Ophthalmologica*. 2014;232:10–27. [PubMed: 24751584]
  45. Rajpal RK, Wisecarver CB, Williams D, Rajpal SD, Kerzner R, Nianiaris N, et al. Lasik xtra provides corneal stability and improved outcomes. *Ophthalmol Ther*. 2015;4:89–102. [PMCID: PMC4675731]
  46. Kanellopoulos AJ, Kahn J. Topography-guided hyperopic LASIK with and without high irradiance collagen cross-linking: Initial comparative clinical findings in a contralateral eye study of 34 consecutive patients. *J Refract Surg*. 2012;28:S837–40. [PubMed: 23447898]
  47. Tan J, Lytle GE, Marshall J. Consecutive laser in situ keratomileusis and accelerated corneal crosslinking in highly myopic patients: Preliminary results. *Eur J Ophthalmol*. 2014;25:101–7.
  48. Alhayek A, Lu PR. Corneal collagen crosslinking in keratoconus and other eye disease. *Int J Ophthalmol*. 2015 Apr 18;8(2):407-18. doi: 10.3980/j.issn.2222-3959.2015.02.35.

In Moderate to Severe Dry eye condition,



**START EARLY**  
**START RIGHT**

**IMUDYOPS** <sup>Preservative free</sup>  
Cyclosporine Eye Drops 0.05%  
**Singules**

*Treat beyond the surface*

**World's 1<sup>st</sup> Micro-emulsion Technology**

Ensures particle size of less than 1 micron



Reference: Cipla Data on file



In VKC,\*

**IMUDYOPS DS** <sup>Preservative free</sup>

Cyclosporine 0.1%



\*Vernal Keratoconjunctivitis

"For any further information, please contact"

**Cipla**

**Cipla Ltd. Regd. Office:**

Cipla House, Peninsula Business Park, Ganpatrao Kadam Marg,  
Lower Parel, Mumbai 400 013, INDIA

Website: [www.cipla.com](http://www.cipla.com)



INDIAN SOCIETY OF CORNEA AND KERATOREFRACTIVE SURGEONS

INDIAN SOCIETY OF CORNEA AND KERATOREFRACTIVE SURGEONS (LIFE MEMBERSHIP APPLICATION FORM)

Name (BLOCK LETTERS).....

S/D/W/of.....DOB(dd/mm/yy).....

Qualifications.....

ADDRESS

Correspondence.....

.....Phone.....

Hospital / Clinic.....

.....Phone.....

Mobile.....FAX.....

Nationality ..... Email.....

I wish to become a Life Member of the 'Indian Society of Cornea and Keratorefractive Surgeons' and shall abide by the Constitution and Rules & Regulations of the Society.

Please Find enclosed my membership Fees of Rs. \_\_\_\_\_(in words) Rs. \_\_\_\_\_ By Cash/Cheque/DD No. \_\_\_\_\_ dt. \_\_\_\_\_ drawn on \_\_\_\_\_.

Also enclosed is a brief C.V. (max 2 pgs) highlighting my training and experience in Cornea specialty&/or Keratorefractive surgery & degree Certificate.

I declare that the information provided by me as above is correct

(Signature of Applicant)

\_\_\_\_\_

FOR OFFICIAL USE

Dr. \_\_\_\_\_ has been admitted as a Life Member of the Indian Society of Cornea and Keratorefractive Surgeons in the Governing Body Meeting held on \_\_\_\_\_ His/Her membership no. is \_\_\_\_\_, Membership Fee received by Cash/Chq/DD no. \_\_\_\_\_ dated \_\_\_\_\_ drawn on \_\_\_\_\_

Address: ISCKRS Secretariat - Dr. Rajesh Sinha, 474, 4th Floor, R.P. Centre for Ophthalmic Sciences AIIMS Life Membership fee is Rs. 3,000/- payable by local (Delhi) Cheque or by Demand Draft payable at Delhi in favour of "Indian Society of Cornea and Keratorefractive Surgeons".

## **JOIN ISCKRS**

The Indian Society of Cornea & Kerato-refractive Surgeons (ISCKRS) has been active, dedicated and committed to the advancement of the understanding of diseases of the Cornea, External Eye & Refractive Surgery. All admitted members of ISCKRS shall be "Life Time Members" of the society, enjoying benefits, bound by terms laid in the Constitution & By Laws of ISCKRS.

To Become Member, please visit <http://www.isckrs.org/membership.aspx>

## **BENEFITS**

All admitted members of the society shall be Life Time Members of the society, enjoying benefits, bound by terms laid down in the Constitution & By-Laws of ISCKRS. All Life Member of ISCKRS shall enjoy the following benefits

1. ISCKRS Newsletter
2. Hands on Training Courses
3. Skill Transfer Courses
4. Certification of Training
5. Clinical Recommendations and Guidelines: Management Protocols
6. Online Journal Access
7. Online Photo Gallery
8. Online Video Gallery

# OSMODROPS

CMC 0.5% + Glycerine 0.9%

EDGE OVER HYDRATION



## WHEN DRY EYE PROGRESSES CHOOSE OSMODROPS TO PROVIDE AN EDGE OVER HYDRATION

- Formulated with CMC 0.5%, Glycerine 0.9%, L-carnitine and Erythritol for osmoprotection
- Mixture of 2 grades of BLANOSE CMC to provide the best hydration and retention time.
- 3-piece Metered-Dose Dropper to prevent wastage of drops and provide cost-effective therapy.

*“For any further information, please contact”*

**Cipla**

**Cipla Ltd. Regd. Office:**

Cipla House, Peninsula Business Park, Ganpatrao Kadam Marg,  
Lower Parel, Mumbai 400 013, INDIA  
Website: [www.cipla.com](http://www.cipla.com)

**Cipla**  
**FORESIGHT™**  
vision becomes reality

**Introducing****QUEEN** of **QUINOLONES**

# Gatiquin HS

Gatifloxacin 0.5% EYE DROPS

## The Future of Infection Control

### Gatifloxacin Higher Strength Benefits

- Improved efficacy<sup>1</sup>
- Helps prevent development of resistance<sup>2</sup>
- Maintains the safety of the 0.3% formulation<sup>2</sup>
- Allows for a less frequent dosing regimen<sup>2</sup>



References:

1. <http://optometrytimes.modernmedicine.com/print/147790> Last accessed on 2nd January 2016

2. Heller W., Cruz M., Bhagat YR., Leon JM., Felix C., Villanueva L., et al. Gatifloxacin 0.5% administered twice daily for the treatment of acute bacterial conjunctivitis in patients one year of age or older. *Journal Of Ocular Pharmacology And Therapeutics* 2014: 1-8

*"For any further information, please contact"*

**Cipla****Cipla Ltd. Regd. Office:**

Cipla House, Peninsula Business Park, Ganpatrao Kadam Marg,  
Lower Parel, Mumbai 400 013, INDIA

Website: [www.cipla.com](http://www.cipla.com)