Guest editorial
AIOS-profile and prospects

Cover story
Limbal stem cells

Major reviews
Amniotic membrane transplants
Fungal corneal ulcer
Anti VEGF in retina

Biostatistics made easy
Introduction

Surgical corner
Small pupil cataract surgery

Common tests simplified
FFA

Original articles
Early PPV in PDR
Ocular lymphoma
Iatrogenic breaks during vitrectomy

Brief reports
Bilateral ACG with topiramate therapy
OCT in tamoxifen retinopathy
Challenges in viral keratitis
Best disease
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Contents

183 Editorial

184 Guest Editorial
   NSD Raju

Cover Story

186 Limbal Stem Cells
   Anil Radhakrishnan, Devi Manilal

Major Review

191 Amniotic Membrane in Ophthalmology: Current Indications
   Vinay S Pillai

199 Fungal Keratitis
   V Sujith Nayanar, Vanathi

212 Anti VEGF in Retinal Diseases – An Evidence Based Review
   Lakshmipriya, Gopal S Pillai, Natasha Radhakrishnan

Biostatistics Made Easy

219 Introduction to Biostatistics in Medical Research
   K R Sundaram

Surgical Corner

226 Cataract Surgery in Eyes with Small Pupil
   Sreeni Edakhlon, Jaison V A, Srikant Karat

Common Tests Simplified

228 Fundus Fluorescein Angiography
   Natasha Radhakrishnan
Original Articles

231 Long Term Visual Outcome of Type 2 Diabetic Patients Undergoing Early Vitrectomy and Endolaser for Severe Vitreous Hemorrhage
Sonia Rani John, Meena Chakrabarti, Arup Chakrabarti

235 Clinical Profile and Treatment Outcome of Ocular Lymphoma – A Single Institution Experience - Aparna C Varghese, Ani Sreedhar, Anju S Raju

240 Iatrogenic Peripheral Retinal Breaks in 20-G Pars Plana Vitrectomy
Ashok Nataraj, Tony Fernandez, Freddy T Simon, Gigi Augustine

247 Outcome of Therapeutic Keratoplasty in Fungal Keratitis
Aneeta Jabbar Reesha K R, Seema K M

Brief Reports

252 Bilateral Acute Angle Closure Glaucoma due to Topiramate
Thomas Arun Varghese

254 Spectral Domain Optical Coherence Tomography and Fundus Autofluorescence Findings in Tamoxifen Retinopathy – A Case Report
N Sandhya

257 Challenges in Viral Keratitis
Rose Mary Tomy

260 A Case Of Multifocal Best disease
Biju John C, MD DNB FRCS, Arya A R, MS

264 Journal Review

268 Book Review

270 PG Corner

272 Spot Diagnosis

273 Instruction to Authors
Each issue of the KJO is indeed like a baby born, it requires elaborate preparations, goes through mid phase slumber and is finally brought out with a lot of push and pull amidst cries of support and anguish. This edition is no different and I pray, you look at this issue with kind eyes. This is the third issue that the current editorial board is bringing out and I hope you all would have got your copies by the time of our state conference, Drishti, this time at Trivandrum. Again this is a mixed bag issue, slightly skewed towards cornea, as all the corneal surgeons of Kerala together helped us out this time.

The first article of this issue is given by none other than our beloved President of the All India Ophthalmological Society, Dr NSD Raju. In his Guest Editorial, he goes through the profile of AIOS, a legacy we should all be aware of, and his plans to lunge the Indian Ophthalmology forward, all around the world. This is a must read for all Ophthalmologists who should be well aware of the future prospects, the AIOS has to offer.

The cover story, this time, is about stem cells, lots of hopes and promises are bestowed upon these primitive cells which we think can transform to any type of cell in the human body. Limbal stem cells are a widely researched area in the stem cell world and definite disorders due to its deficiency are in record. Limbal stem cells provide the stem cell interest group, a lot of information as they are almost the first stem cells that are transplanted with huge success. In this article, we look at the physiology and anatomy of limbal stem cells, its clinical significance and deficiency states.

The first major review is an out of ordinary, but important topic. We go into Amniotic membrane transplantation in Ophthalmology, another key support strategy which provides a lot of biologic support to corneal healing and recovery. This detailed article will give a deep insight to the current indications and applications of amniotic membrane in the eye. A very useful article for postgraduates, getting an exhaustive review in capsule form.

Out of ordinary to most ordinary- Fungal keratitis has never failed to raise any eyebrow, a savage adversary to any practicing Ophthalmologist, it has caused sleepless nights to scores of doctors and robbed sight off hundreds of patients- A difficult case to treat. This article has lucidly described the etiology, pathology, clinical features, diagnosis and management of fungal corneal ulcer. Basics and advances are dealt with in balance.

The last major review is on a revolution in Ophthalmology which has happened over the last 5 years, Anti VEGF in retinal diseases. This is fast beating cataract surgery as the most common intraocular procedure performed in human eye. When any patient requires only two cataract surgeries, each eye of a patient may require more than a dozen injections, just to maintain vision. Even then it has become the fast and the furious treatment in eye diseases and this article looks at evidence in the management of retinal diseases.

Prof Sundaram, in his elegant article, teaches us the basics of Biostatistics, study designs and analytical methodologies adopted by statisticians to prove and disprove hypothesis. He has, with the help of numerous examples tried to improve our basic concepts in the field of statistics.

In the surgical side, we have three doyens from North Kerala joining hands to tackle small pupil, a common problem that all cataract surgeons face at one time or the other. The videos demonstrate the different techniques, all young cataract surgeons can adopt for managing the small pupil. This article is best read along with the video on youtube.

Fundus fluorescein angiography is the common test that we are trying to simplify this time. The equipment, technique, phases and findings are described. Causes of hypo and hyperfluorescences, auto and blocked fluorescences are described along with pictures. This article will provide the post graduate as well as comprehensive Ophthalmologist with optimal knowledge in the field of FFA. It is important to know which patients require the test and what would we want to know in that patient.

Original articles and brief reports, Journal review and book review, PG corner and Spot diagnosis brings the rear end of our Journal. Let me once again thank you in spending time with the journal and would look forward to the advises that you give me after each of these babies are born. Please contribute articles to the journal.

Jai KSOS

Dr Gopal S Pillai
Editor KJO
All India Ophthalmological Society (AIOS) –
A Profile and Future Prospects

One of the biggest congregations of ophthalmologists in the world today, the All India Ophthalmological Society with more than 16000 members on its roll is heading towards greater and greater heights of glory in terms of professional expertise, academic excellence, organisational solidarity and social commitments. Certainly, Indian ophthalmology is all poised for a giant leap to the top position in the global atlas of Ophthalmology.

Founded in 1938 with a small group of ophthalmologists spread across the country, the society has since grown in strength and stature and achieved a global recognition and status thanks to the unyielding efforts and endeavours of the leaders of the ophthalmic profession in the country and their mighty contribution to the Society. It indeed is a great prestige and proud privilege to be a member of All India Ophthalmological Society. The numerous benefits that accrue from the membership of AIOS speak volumes for the comprehensive care and support being provided to its members. The Indian Journal of Ophthalmology is one of the best peer reviewed ophthalmology journals in the world today. Periodic publication of Continuing Medical Education series by the Academic and Research Committee keep the members updated on the current concepts, newer technology and recent advances in ophthalmology. The FAICO fellowship Program of AIOS Collegium is a most unique one and has established itself as one of the most covetable super specialty qualifications in the country. The innumerable awards, accolades and fellowships offered by AIOS are certainly a great incentive to ophthalmologists especially the young and the enterprising ones. The annual conference of the Society is one of the biggest scientific conventions in the world, drawing more than 7000 delegates from across the country and abroad. The contents of the whole scientific programme are compiled into Proceedings and published along with audio and video support. These publications by AIOS Proceedings are a great draw and attraction to the members. The Society also organises online Continuing Medical Education Programs in the form of Webinars, Webcasts, online seminars, WEB video library, Podcasts and the likes, to name a few. The Family Benefit Scheme - AIOS, provide social security and support to the member’s family, and the newly introduced Professional Protection Scheme takes care of the medico legal aspects including financial support in cases of litigation that may arise in course of our professional practice.

The AIOS is firmly committed to make Indian Ophthalmology as one of the best professional bodies in the world, and of course, our Society is definitely forging ahead to this most enviable status. In fact our Society is envisaging certain measures that would beget an upsurge and fillip to our great organisation in the next decade during which period the AIOS foresees to launch and implement many innovative and novel measures to take Indian Ophthalmology to the next higher level of achievements and provide maximum support and backing for its members. AIOS will also play a pivotal role in all the schemes initiated by the government in the field of prevention of blindness programmes and in the implementation of standardised ophthalmic care system in the country. This will help the government to set priorities in the ophthalmic healthcare system and evolve a methodology best suited for the needs of the country. An integration of public and private ophthalmic resources is absolutely essential and for this, and the All India Ophthalmological society will provide the government all the necessary support and help in the incorporation of this new approach into the ophthalmic health care system in the country.

The professional excellence and expertise of Indian ophthalmologists are unquestionably of a very high standard and certainly on par with those in developed countries. However, for some reason or the other, we have not been that successful in projecting a true image of Indian Ophthalmology to the world at large. It will be the endeavour of Team AIOS to devise and evolve a suitable strategy to achieve the global status which we so richly deserve. Henceforth the AIOS will have official representation at all major international conferences where an excellent Resource Centre of AIOS will provide a true image of Indian ophthalmology. We also propose to introduce international membership of AIOS to ophthalmologists from other countries thus opening up better avenues for interaction and professional partnership. Organising mutual exchange and training programmes is another appropriate and effective approach. In fact we have our fellows of LDP training programme and in the proposed mutual exchange and training programmes they will have

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an excellent opportunity to interact with their counterparts abroad and contribute significantly to promoting AIOS and Indian Ophthalmology overseas.

The increasing incidence of blindness from Diabetes has been a cause for major concern for us. AIOS has already outlined a nation wide approach to this ever increasing menace of ocular morbidity and blindness which are preventable and treatable. Over the next decade we will endeavour our best to eradicate this avoidable blindness due to diabetes and its complications. To achieve this, a well planned systematic approach and methodology are essential. In this regard AIOS has an important and crucial role to play. Nation wide screening for diabetes and diabetic retinopathy is the most important prerequisite for the successful implementation of this vital project. Epidemiological aspects also will be a useful adjunct research methodology for the evaluation of the incidence of diabetic retinopathy. The next essential step is to identify and network the tertiary and teaching institutes in the country and utilize their expertise and services preventable blindness. The AIOS has already suggested setting up of tele ophthalmology centres across the country in all remote rural areas with networking facility to major ophthalmic institutions to transmit ophthalmic images to these major centres. This will be a key measure in the implementation and completion of our campaign against diabetic blindness. In fact this scheme has already been implemented at various centres. But we need to establish this system all over the country to make this a successful venture. Mobile tele ophthalmology centres are also being made available to supplement the screening procedure to detect and refer patients with diabetic retinopathy for appropriate treatment.

Combating childhood blindness has been identified as the most cost effective health-care intervention. Inadequate population-based data on prevalence and causes of blindness in children, shortage of paediatric eye care professionals and fragmentation of paediatric eye care services have all contributed to this vexed problem that has assumed a formidable dimension, particularly in developing countries like India. AIOS is taking up this most challenging issue on a war footing. Of the causes of childhood blindness, congenital anomalies, retinopathy of prematurity and cataract are the major ones. Special screening programmes, creating awareness amongst medical personnel, intensive training to concerned medical and paramedical and administrative staff are all methods identified by AIOS to combat childhood blindness. The next decade will witness a paradigm shift in the management and prevention of Childhood blindness in India. In the ophthalmic education curriculum, All India Ophthalmological Society is very keen on streamlining the postgraduate and residency courses. Lack of uniform syllabus on practical training has been a major problem. AIOS is suggesting an improved methodology to rationalize and restructure the ophthalmic post graduate educational system to suit the needs of the country. The residents emerging out of this reorganised educational system will be competent and capable, and be able to handle surgical and medical ophthalmic problems without the need for immediate further practical training. To evolve this new improved system of education, all post graduate ophthalmic institutes must be uniformly equipped with a standardized infrastructure with all necessary equipments, sufficient clinical materials and adequate number teaching personnel. As proposed and recommended by AIOS a time bound strategy in a phased manner is already in the pipeline to streamline the ophthalmic post graduate educational system in the country.

The Team AIOS is very confident of achieving these ideal goals for the benefit of its members in the near future itself and in that process; we will also be strengthening and supporting ophthalmic health care system in the country.

Dr. NSD Raju is the current President of the All India Ophthalmic Society. Dr. Raju is only the second Ophthalmologist from Kerala to become the President of All India Ophthalmological Society. He is currently the Director of Ranjini Eye Hospital, Cochin, an ISO certified NABH accredited Eye Hospital. He is a pioneer in Intra Ocular Lens Implant Surgery in India. Dr. Raju has been an active member of the AIOS for the last 30 years.
Introduction
The corneal epithelium contain stem cells which represent the proliferative reserve. Corneal epithelial stem cells are localised exclusively in the basal limbal epithelium. Loss or malfunction of stem cells does not permit maintenance or regeneration of the corneal epithelial mass but leads to conjunctivalisation of the corneal surface. Clinically, several ocular surface disorders such as chemical burns can cause limbal damage and consecutive limbal insufficiency. Treatment for these cells, which can be performed as both autograft and allograft disorders is available only by transplantation of healthy stem cells. Stem cells have certain characteristics like longevity, high capacity of self renewal with a long cell cycle time and a short S-phase duration, increased potential for error-free proliferation, and poor differentiation.

The ocular surface is made up of two distinct types of epithelial cells, constituting the conjunctival and the corneal epithelia. Although anatomically continuous with each other at the corneoscleral limbus, the two cell phenotypes represent quite distinct subpopulations. Stem cells for the cornea reside at the corneoscleral limbus. The limbal palisades of Vogt and the interpalisade rete ridges are believed to be repositories of stem cells. The microenvironment of the limbus is considered to be important in maintaining the stemness of stem cells. Limbal stem cells also act as a “barrier” to conjunctival epithelial cells and normally prevent them from migrating on to the corneal surface. Under certain conditions, however, the limbal stem cells may be partially or totally depleted, resulting in varying degrees of stem cell deficiency with resulting abnormalities in the corneal surface. Such deficiency of limbal stem cells leads to “conjunctivalisation” of the cornea with vascularization, appearance of goblet cells, and an irregular and unstable epithelium. This results in ocular discomfort and reduced vision. Partial stem cell deficiency can be managed by removing the abnormal epithelium and allowing the denuded cornea, especially the visual axis, to resurface with cells derived from the remaining intact limbal epithelium. In total stem cell deficiency, autologous limbus from the opposite normal eye or homologous limbus from living related or cadaveric donors can be transplanted on to the affected eye. With the latter option, systemic immunosuppression is required. Amniotic membrane transplantation is a useful adjunct to the above procedures in some instances. Stem cells are “undifferentiated cells capable of (a) proliferation, (b) selfmaintenance, (c) a large number of differentiated, functional progeny, (d) re-generating the tissue after producing injury, and e) a flexibility in the use of these options.” Stem cells are responsible for ultimate cellular replacement and tissue re-generation.

The X, Y, Z Hypothesis of Corneal Epithelial Maintainence

\[X = \text{proliferation of basal cells} \]
\[Y = \text{centripetal movement of cells} \]
\[Z = \text{cell loss from the surface} \]

\[X + Y = Z \]
Characteristics of Stem Cells
Stem cells are responsible for ultimate cellular replacement and tissue regeneration.

1. Stem cells are poorly differentiated; the cytoplasm of stem cells appears primitive and contains few differentiation products.

2. Stem cells have a high capacity for self-renewal, with an increased potential for error-free proliferation and cell division.

3. Error-free proliferation is essential, as any genetic error at the level of stem cells will continuously and permanently pass on to the whole clone of cells, resulting in abnormal differentiation and cellular dysfunction.

4. Stem cells have a long life span, which might be equivalent to the life of the organism in which they reside.

5. Stem cells have a long cell cycle time or slow cycling (which indicates low mitotic activity).

6. Cell division within stem cells can be intrinsically asymmetric, asymmetric only with regard to daughter cell fate, or symmetric. When cell division is obligatorily asymmetric, one of the daughter cells remains as its parent and serves to replenish the stem cell pool, whereas the other daughter cell is destined to divide and differentiate with the acquisition of features that characterize the specific tissue. On the other hand, the asymmetry in division may be determined by the local environment, which induces otherwise similar daughter cells to behave differently. Finally, all divisions of the stem cell may be symmetric, but are “selfrenewing” only half the time.

Stem Cells and the Corneal Epithelium.
The corneal epithelium exists in a state of dynamic equilibrium, with the superficial cells being constantly shed into the tear pool. Eyelid blinking causes terminal differentiation of cells, coupled with cell death by apoptosis and cell loss via desquamation.

Identification and Location of Limbal Stem Cells
The methods for identifying corneal epithelial stem cells are indirect. Direct markers for stem cells have not been established, but there is clinical and experimental evidence supporting the location of corneal epithelial stem cells at the limbal region. Davanger and Evensen in 1971 proposed the concept that epithelial cells in the limbal region are involved in the renewal of corneal epithelium. In healed eccentric corneal epithelial defects in heavily pigmented eyes, they observed pigmented epithelial migration lines (cells) that migrated from the limbal region toward the central cornea. The suggested that the limbal papillary structure (palisades of Vogt) serves as a generative organ for corneal epithelial cells. Later, experimental studies by Schermer et al and Cotsarelis et al confirmed that the source of cell proliferation and migration after a corneal epithelial defect is the scleroconeral limbus. The current evidence of the limbal location of stem cells. The limbal basal epithelium contains cells that exhibit the proliferative characteristics of stem cells. The limbal location of corneal epithelial stem cells can account for the relative preponderance of limbal neoplasms and the scarcity of corneal epithelial tumors, assuming that neoplasms arise primarily from relatively “undifferentiated” cells.

1. The limbal basal epithelium contains the least differentiated cells of the corneal epithelium. Epithelial cells contain different types of keratins, some of which indicate a high level of differentiation, whereas others are found mostly in less differentiated cells. The differential expression of keratins allows the separation of cell populations within the corneal epithelium according to their level of differentiation. No markers have not been identified to label limbal epithelial stem cells. The 64 KD keratin K3 indicates a cornea-specific type of differentiation. It was observed that keratin K3 exists in the suprabasal epithelium of the limbus and the entire corneal epithelium, but is not expressed in either the limbal basal epithelium or the adjacent bulbar conjunctiva. This observation led to the hypothesis that the limbal basal epithelium lacks a differentiated cornea-type phenotype, and therefore contains the least differentiated cells of the epithelium, i.e., stem cells. The limbal basal epithelium also lacks the expression of the corneal-specific keratin K12, which is expressed in both the suprabasal limbal epithelium and the entire corneal epithelium. Other studies that evaluated the expression of different proteins and indicators of a relatively undifferentiated phenotype provided further evidence for the low level of differentiation of limbal basal epithelium.

2. The limbal basal epithelium contains cells that exhibit the proliferative characteristics of stem cells. Limbal basal epithelial cells have a higher proliferative potential in culture than central and peripheral corneal epithelial cells. Limbal basal cells respond to central corneal wounds and to tumor-promoting agents by undergoing higher proliferation than central corneal epithelial cells, which terminate proliferation-initiating differentiation.

Labeling studies have demonstrated that the mitotic index of the corneal epithelium tends to be higher toward the periphery, suggesting that the peripheral corneal basal cells
are more active in DNA synthesis. Cotsarelis et al found that tritiated thymidine was incorporated for long time intervals only into limbal basal cells. This labeling indicated that these cells exhibited a long cell cycle. These cells are also resistant to the induction of differentiation. Growth factors, retinoic acid, and calcium have been shown to affect the limbal and central corneal epithelial cell types differently.

3. Further support for the limbal location of corneal epithelial stem cells is derived from experimental studies and clinical observations of abnormal corneal epithelial wound healing when the limbal epithelium is partially or completely removed. These studies produced a spectrum of corneal surface abnormalities characterized by conjunctival epithelial ingrowth (conjunctivalization), vascularization, and chronic inflammation, which indicated limbal stem cell deficiency. The conjunctival source of the epithelial ingrowth was proved by immunofluorescent staining with monoclonal antibodies and by the detection of goblet cells with impression cytology. The minimal anchorage of the corneal epithelium can account for the relative preponderance of limbal neoplasms and the scarcity of corneal epithelial tumors, assuming that neoplasms arise mainly from relatively "undifferentiated" cells.

5. A mathematical analysis of the kinetics of maintenance of the corneal epithelial mass confirms that the corneal epithelium can be maintained by cellular proliferation originating from limbal stem cells without contribution of the adjacent conjunctiva.

SUMMARY
Stem cells are located exclusively in the limbal basal epithelium. The specific location of corneal epithelial stem cells in the limbus provides several functional advantages. Because central corneal epithelium has to be transparent, its basal cells are devoid of pigment and, consequently, are highly susceptible to solar damage. Basal cells in the limbal region do not have this constraint; they are heavily pigmented and, thus, are well protected. The transparency of the cornea also dictates a smooth epithelial-stromal junction. This minimal anchorage renders corneal epithelium susceptible to physical shearing. In contrast, limbal epithelium is very resistant to shearing forces and displays a highly undulating epithelial-stromal junction. This would give a natural advantage for retention of stem cells in the face of environmental onslaughts.

Limbal Stem Cell Deficiency

CAUSES
The etiology of limbal stem cell deficiency can be related to an insufficient stromal microenvironment to support stem cell functions, such as aniridia. However, most commonly, stem cell deficiency is related to external factors that destroy the limbal stem cells, such as chemical or thermal injuries, ultraviolet and ionizing radiation, Stevens-Johnson syndrome, advanced ocular cicatricial pemphigoid, multiple surgeries or cryotherapies, contact lens wear, or extensive microbial infection.

CLINICAL PRESENTATION
Limbal stem cell deficiency is associated with conjunctivalization of the cornea. Biomicroscopic examination at the slit-lamp may show the corneal epithelium to have a dull and irregular reflex and to be opaque and of variable thickness. Limbal stem cell deficiency can be complicated with recurrent and persistent epithelial defects, superficial corneal vascularization, scarring, calcification, ulceration, melting, and perforation of the cornea. Because the conjunctival epithelium is more permeable than corneal epithelium, conjunctivalized corneal surfaces frequently are stained abnormally by fluorescein.

Partial Limbal Stem Cell Deficiency
When the visual axis is covered with epithelial cells with corneal phenotype, vision is usually good, and if the patient is otherwise asymptomatic, only conservative treatment is indicated. Topical lubrication may be helpful to prevent corneal epithelial erosions. For patients with subtotal corneal-limbal-conjunctival epithelial defects, the occurrence of partial stem cell deficiency can be anticipated and prevented by close monitoring. Healing of the cornea and conjunctiva should be allowed to occur by their respective phenotype of cells. Any attempt by the conjunctival epithelium to cross the limbus should be checked by mechanically scraping or brushing off the advancing edge of conjunctival epithelium. This may need to be repeated on a few occasions, before the circumferentially migrating limbal epithelial cells have met and re-established the limbal barrier. Patients with partial stem cell deficiency with irritation, decreased vision (usually when visual axis is covered with conjunctival epithelial cell phenotype) or persistent epithelial defects may require surgical inter-vention.

In partial (and total) stem cell deficiency, sector limbal transplantation (using autologous or homologous tissue) has been reported to give beneficial results. For partial stem cell deficiency (conjunctivalization of the cornea), mechanical debridement of conjunctival epithelium from the corneal surface, under topical anesthesia must be done. The key to the success of this technique is close monitoring of the patient after the debridement to ensure that healing of the denuded corneal surface occurs from the remaining corneal epithelium and not from the conjunctival epithelium.
Mechanical debridement would not be effective in patients with total limbal stem cell deficiency. The presence of an amniotic membrane might make it difficult to monitor the migration of conjunctival epithelium, which often occurs beneath the membrane, on the debrided area. Moreover, if this were to occur, the presence of the membrane would impede any effort to remove the conjunctival epithelium from the corneal surface. However, at present this is a theoretical consideration, as there is no study comparing the efficacy of mechanical debridement, partial limbal transplantation and amniotic membrane transplant in pa-tients with partial limbal stem cell deficiency. Good visual results can be obtained if the treatment ensures that the visual axis is covered with cells of corneal phenotype, whereas other areas may still be covered by cells of conjunctival phenotype.

Diffuse Limbal Stem Cell Deficiency

Patients with stem cell deficiency affecting the whole corneal surface require limbal transplantation to restore the corneal surface. A variety of techniques have been reported for limbal stem cell transplantation. All the procedures share the goal of transplantation of a new source of epithelium for a diseased ocular surface after the removal of the host’s altered corneal epithelium and pannus. After successful transplantation, the host’s cornea (or grafted cornea) will be permanently covered by epithelium from the donor. Although all techniques used in stem cell transplantation are similar in principle, the source of donor stem cells can vary. When the donor tissue is obtained from the fellow eye; it is termed a limbal autograft, which is used in cases of unilateral disease. Tissue can also be obtained from a living related donor or cadaver donor (whole globe or corneoscleral disk); this limbal allograft can be used when both eyes are affected. Autologous tissue should not be used in patients who have an asymmetric presentation of a known bilateral condition, like Stevens-Johnson syndrome with one eye severely affected and following chemical injury, where use of autologous tissue from the other eye should be approached with caution. Limbal autografts and tissue from living related donors should not be used in the immediate postinjury period. Such lenticules may be destroyed in the ongoing acute inflammatory response. If stem cell transplantation is considered necessary in the immediate postinjury period, cadaveric donor material should be used.

Limbal transplantation procedures vary, depending on the carrier tissue used for the transfer of the limbal stem cells. Carrier tissue is needed in limbal transplantation because it is not possible to transfer limbal stem cells alone. Limbal transplants have included either conjunctiva (conjunctival limbal graft, the peripheryal cornea is also invariably included), cornea (keratolimbal graft), or both as a carrier tissue for limbal stem cells. Limbal stem cells can also be transplanted with penetrating keratoplasty with homologous oversized grafts and with central corneal transplantation of an eccentrically trephined corneolimbal transplant. Conjunctival transplantation is not useful for treating limbal stem cell deficiency. In all eyes in which simultaneous removal of limbal and corneal epithelia had caused corneal conjunctivalization and neovascularization, limbal transplantation significantly reduced the area of neovascularization and allowed restoration of the corneal epithelial phenotype. On the contrary, conjunctival transplantation was not successful and the corneas developed progressive vascularization with continuous expression of a conjunctival phenotype. As for partial stem cell deficiency, even in total stem cell deficiency, regardless of whether allo- or autograft material is used, it is important to monitor the patient closely during the healing process and to ensure that conjunctival epithelium does not encroach onto the corneal surface. Re-epithelialization of the cornea should be allowed to occur from cells derived from the transplanted limbal tissue only. After surgery, autologous serum eyedrops have been used to promote corneal epithelialization. Amniotic membrane transplantation combined with limbal transplantation has been successfully used in patients with diffuse limbal stem cell deficiency and severe ocular surface disease, including Stevens-Johnson syndrome, advanced ocular cicatricial pemphigoid and chemical and thermal burns. Alternatively, autologous limbal-conveal epithelium can be cultured on amniotic membrane and used for corneal surface reconstruction. Amniotic membrane transplantation may be helpful in restoring the abnormal basement membrane and damaged stromal matrix, possibly restoring the normal microenvironment of stem cells and transient amplifying cells. The use(s) of amniotic membrane in the management of stem cell deficiency and ocular surface reconstruction is still evolving. It may act as a biological bandage wherein epithelial healing occurs under the membrane, or as a basement membrane (stroma) transplant, in which the healing epithelium migrates on the surface of the transplanted membrane, which is believed to be incorporated into the ocular surface. Various growth factors produced by the membrane may promote epithelial healing.

Symptoms

The clinical symptoms of limbal deficiency may include decreased vision, photopho-bia, tearing, blepharospasm, and recurrent episodes of pain (epithelial breakdown), as well as a history of chronic inflammation with redness. In some patients the limbal deficiency may be subclinical and may progress eventually to an overt stage, as the stem cell population depletes further, over time. Limbal deficiency may be localized (i.e., partial) or diffuse (i.e., total). In localized limbal deficiency, some sectors of limbal and corneal epithelium are normal. Areas of corneal epithelium with normal (corneal) and conjunctivallike phenotype can usually
be differentiated clinically. The healing of large corneal, limbal, and conjunctival epithelial defects are by a preferential circumferential migration of small tongue-shaped sheets of epithelial cells arising from either side of the remaining intact limbal epithelium.

DIAGNOSIS
Diagnosis of limbal stem cell deficiency is crucial because patients with these abnormalities generally are poor candidates for conventional corneal transplantation.

Lamellar or penetrating keratoplasty provides only a temporary replacement of the host’s corneal epithelium and does not permanently reconstitute the limbal function.

Because vascularization and inflammation also can be seen in other corneal diseases, “conjunctivalization” is the most reliable diagnostic sign of limbal deficiency.

This phenomenon can be detected biomicroscopically and with fluorescein stain. Another feature is loss of the limbal palisades of Vogt, but it should be remembered that the palisade architecture of the limbus is not uniform through the circumference of the limbus. The presence of distinct palisade architecture in some segments and a complete absence in others is consistent with normal limbal function. However, the loss of the palisades of Vogt after an insult, in an area known to have the palisade architecture before the insult, is very indicative of stem cell loss. Confirmation is provided by the use of impression cytology, which can detect goblet cell–containing conjunctival epithelium on the corneal surface and differentiate it from other abnormalities, such as conjunctival squamous metaplasia.

References
Amniotic Membrane in Ophthalmology: Current Indications

Introduction

Amniotic membrane transplantation (AMT) in ocular surgery has become widespread and is used for a variety of ocular surface problems. The first therapeutic use of amniotic membrane (AM) was successfully achieved by Davis1 in 1910 for skin transplantation and the first ocular indication for AM was suggested by de Rotth2 in 1940 following successful treatment of a chemical burn of the ocular surface. But it was not until Juan Batlle’s report in 1992 that it re-emerged as an important modality of treatment.

Structure of amniotic membrane

AM is the inner avascular layer of the three-layered foetal membrane. It is translucent and has an inner layer of epithelial cells on a basement membrane. This is connected to a thin connective tissue membrane by filamentous strands.3 Outside the amnion is the chorion laevae comprising of connective tissue containing the fetal (chorioallantoic) vessels. The outermost layer of the fetal membranes, the decidua capsularis, is the only component of the fetal membranes of maternal origin and is composed of modified endometrium.

Histologically the amnion is a 0.02 mm to 0.5 mm five-layered membrane, composed of three basic layers.

• Epithelial monolayer - single layer of cuboidal cells with a large number of microvilli on the apical surface.
• Thick basement membrane - thin layer composed of a network of reticular fibers. Histochemically the basement membrane closely resembles that of the conjunctiva.4 This compact layer contributes to the tensile strength of the membrane.
• Avascular, hypocellular stromal matrix - is the spongy layer.

Preparation of amniotic membrane

Amniotic membrane is obtained from donors undergoing Caesarean section, who are negative for communicable diseases including HIV, hepatitis and syphilis. There are different protocols for the processing and storage of AM.5, 6 According to Kim et al. 6 the placenta is cleaned with balanced salt solution containing antibiotics (50 mg/ml penicillin, 50 µg/ml streptomycin, 100 mg/ml of neomycin as well as 2.5 mg/ml of Amphotericin B) under sterile conditions. The amnion is separated from the chorion by blunt dissection. The separated membranes are cut in different sizes placed on nitrocellulose paper strips with the epithelial side up. Dulbecco Modified Eagles Medium/glycerol (1:1) is used for cryopreservation and the tissues are frozen at -80 degrees until further use.7,8,9,10 Human AM deprived of amniotic epithelial cells by incubation with EDTA when freeze dried, vacuum packed and sterilized with gamma-irradiation at 25kGy retained most of the physical, biological and morphologic characteristics of cryopreserved AM.11 Fresh AM can also be used and has been noted to function as well as preserved AM when transplanted onto the ocular surface.12 Concerns with fresh AM is that dual serological testing of the donor (at the time of harvesting and 6 months later) which eliminates the slightest risk of disease transmission is not possible. The time interval from tissue procurement to transplantation is short. And so patients have to be brought to the hospital at a short notice unlike with preserved AM, which allows more flexibility in scheduling surgery.12 In addition wastage of unused tissue with non-preserved AM, as opposed to frozen AM where many grafts of varying sizes can be prepared from one placenta and stored, is high. The drawback with the preserved AM is the need for a -70 o refrigerator, which precludes its use outside big institutions.

Mechanisms of action of amniotic membrane

The structural integrity, transparency and elasticity of the amniotic basement membrane make it an ideal tissue replacement for ocular surface reconstruction.

AM has been shown to promote epithelial cell migration, adhesion and differentiation and also to support the growth of epithelial progenitor cells by prolonging their lifespan, maintaining their clonogenicity and preventing epithelial cell apoptosis.13 This action produces the beneficial effect of AMT in persistent epithelial defect (PED) with stromal ulceration.14 It is well established that when AM is used as a substrate for culturing cells from limbal explants, it favours epithelial cell growth and maintains their normal morphology and differentiation. This cultured epithelium can be transplanted with the AM to reconstruct damaged corneas.15 The AM can also be used to promote non-goblet cell differentiation of the conjunctival epithelium.16

AM contains basic fibroblast, hepatocyte and transforming growth factor (TGF) which modulates proliferation and...
differentiation of stromal fibroblasts. AM stromal matrix, which is rich in fetal hyaluronic acid suppressing TGF-B signaling, proliferation and myofibroblastic differentiation of normal fibroblasts, helps in reducing the scarring response.

AM also suppresses the expression of certain inflammatory cytokines that originate from the ocular surface epithelia, including interleukin 1α, IL-2, IL-8, interferon γ, tumor necrosis factor-β, basic fibroblast growth factor and platelet derived growth factor. It also attracts and sequesters inflammatory cells infiltrating the ocular surface and contains various forms of protease inhibitors giving it the anti-inflammatory properties.

Principles of use of AM in ophthalmology
AMT in ocular surface reconstruction is aimed at providing symptomatic relief, promoting epithelialization, reducing inflammation and scarring. The technique of transplantation is as mentioned below.

Inlay or graft technique: Here AM is tailored to the size of the defect. It is meant to act as a scaffold for the epithelial cells to migrate and then merges with the host tissue. The AM is secured with its basement membrane or epithelial side up to allow migration of the surrounding epithelial cells on the membrane.

Overlay or patch technique: Here AM is used like a contact lens in order to protect the healing surface beneath it. It also reduces inflammation by its barrier effect against the chemical mediators from the tear film. When used as patch the membrane can be secured with its epithelial side up and it either falls off or is removed.

Filling-in or layered technique: Here the depth of an ulcer crater is filled with small pieces of AM and finally a larger graft is fixed to the edges of the defect in an inlay fashion. Over this an AM onlay will help in preserving the deeper layers for a longer duration.

Techniques of amniotic membrane transplantation
Though the preferred surgical orientation of the AM on the ocular surface is with the epithelial side up, depending on the indication of use it can be changed as mentioned above. The identification of stromal surface is by the presence of vitreous-like strands that can be raised with a sponge. AM can be fixed to the ocular surface by various techniques. Till recently it was being sutured in place. But with the availability of fibrin glue, AMT can now be fixed without sutures. This increases the patient comfort and helps in reducing the surgical time significantly.

Clinical uses of AM
Clinically AMT is used for reconstruction of corneal surface, conjunctival surface and also for reconstruction of the ocular surface in cases where the whole surface is damaged.

Corneal surface reconstruction
In stromal ulcerations, a Weckcel sponge or blade is used to remove all cellular debris or exudates from the base of the ulcer and loose surrounding epithelium is debrided using a fine forceps and a straight crescent blade. The AM is then placed over the defect after applying the fibrin glue and ironed out over the defect to achieve good adhesion. The loose AM outside the area of the defect is trimmed. The AM can also be sutured with 10.0 nylon monofilament suture, which are placed circumferentially or parallel to the cut edge of the graft in an interrupted or continuous manner. The suture knots must be cut short and knots buried in corneal tissue. To fill in deep corneal ulcers, descemetoceles or perforations, a multilayered approach where small pieces of AM may be layered into the defect or a single sheet may be folded on itself twice (blanket fold) is used along with a larger patch anchored over the entire defect in an overlay fashion is preferred.

Conjunctival surface reconstruction
After adequate dissection and removal of pathological subconjunctival tissue, bared area is covered with AM. AM is fixed with fibrin glue. If sutures used, then 8-0 or, 9-0 or 10-0 vicryl may be used.

To anchor AM to the fornix, fornix forming sutures may be used. The suture is passed from the fornix through the entire thickness of the lid to the skin and anchored over bolsters outside.

Ocular surface reconstruction
In cases with extensive ocular surface damage seen in severe grades of chemical injury, Steven Johnson syndrome (SJS) etc, a large sheet of AM is placed on the ocular surface and it is first anchored to the lid margin using 8-0 vicryl suture. AM can then fixed to ocular surface with fibrin glue. A continuous encircling 10-0 nylon suture is used to anchor the membrane at the limbus or the peripheral 360 o cornea and multiple interrupted vicryl sutures are placed to attach the membrane to the inner lid surface, beyond the fornix and onto the bulbar conjunctiva. It is then anchored to the fornices with fornix sutures.
Clinical applications:

Cicatrising conjunctivitis:
In cicatrizing conjunctivitis, the success depends on the underlying pathology. Conditions like Stevens–Johnson syndrome and ocular cicatricial pemphigoid where there is progressive cicatrisation the prognosis is poor compared with ‘burnt out’ or long-standing cicatrisation like in chemical burns. The immune-mediated inflammation has to be controlled prior to surgery. John et al. first reported the beneficial effects of AMT in the acute stage of toxic epidermal necrosis. Honavar et al. evaluated the role of AMT as a preliminary step in the sequential management of SJS. They could achieve improvements in the ocular surface, in terms of greater patient comfort, reduced surface inflammation, decrease in the severity of vascularization and absence of recurrent corneal erosions.

Chemical and thermal injury:
In eyes with acute ocular burns AMT helps in pain relief and rapid epithelialization. Performing AMT during the first 7-10 days following acute burns maximizes the effects of the treatment. In severe ocular burns, no definite benefit of AMT over medical therapy alone has been reported. Joseph et al. reported that AMT was not found to be useful in the restoration of the ocular surface in Grade IV burns. It may be because very severe ocular burns results in total destruction of limbal and conjunctival epithelial cells leaving little resource for regeneration.

Associated lid deformities, symblephara and conjunctival foreshortening complicate management of chemical injury in the late stages. In cases of partial limbal stem cell deficiency, Sangwan et al. documented successful outcomes in four patients treated with AM following pannus resection.
**Ex vivo expansion of epithelial cells on the AM**

AM has been established as a carrier for cultured limbal cells for ocular surface reconstruction procedure.\(^{22,33,34,35}\) Advantage of ex vivo expansion is that only a small amount of limbal tissue is harvested from the uninvolved eye. AM acts as a natural substrate for the cell growth and when transplanted gets integrated onto the corneal surface. Its basement membrane contains Type IV collagen and laminin which plays an important role in cell adhesion.\(^ {36}\) Further, it enables easier handling during transplantation.

**Bullous keratopathy**

In bullous keratopathy AMT may be useful in reducing symptoms\(^ {37,38,39} \) and may be attempted in cases with no visual potential or as a temporary measure in patients waiting for corneal transplantation and intolerant to bandage contact lens (BCL). Pires et al\(^ {37} \) described the use of AM in the treatment of bullous keratopathy. However, long-term relief from AMT needs to be studied and compared with other modalities.

**PED and corneal perforations**

Advantage of AMT in PED has been established. In PED, AM serves to provide a basement membrane substrate for the migration and adhesion of epithelial cells when used as an inlay graft. When used as an overlay patch it facilitates epithelialization similar to a BCL and by providing a barrier against inflammatory cells and mediators. The AM, being continuously moistened by tears, provides adequate hydration to the regenerating epithelium and protects it from the abrasive effect of an abnormal palpebral conjunctiva.\(^ {40}\)

Kruse et al\(^ {41} \) described the use of AMT as a possible space filler in cases with thinning of the corneal stroma, to increase stromal thickness by placing the AM one above the other, either with conventional sutures or fibrin glue.\(^ {42}\) However, the early detachment of the membrane despite diligent suturing remains a limiting factor with its use.\(^ {43}\) Su and Lin\(^ {44} \) have described the use of the AM to successfully seal corneal perforations.

**Pterygium surgery**

Pterygium excision with a conjunctival autograft is considered as the gold standard in pterygium surgery. Prabhasawat et al\(^ {45} \) reported the use of AM as an adjunct in pterygium surgery. They reported a recurrence rate of 10.9% for primary pterygium following excision with AMT in a prospective study, which was further reduced to 3% by modifying the treatment.

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Figure 3 A: Post chemical injury with scarring of nasal conjunctiva resulting in restricted abduction along with PED and thinning of corneal stroma

Figure 3 B: Same case in 3A after excision of subconjunctival scar. The defect is covered AM which is glued with fibrin glue. The PED is covered with multilayered AM.

Figure 4: Cheese wiring of the suture which has resulted in loose AM

Figure 4: Residual subepithelial membrane after AMT
surgical technique. They believe that AM could serve as a useful alternative to conjunctival grafts especially when there is a very large conjunctival defect to cover as in primary double-headed pterygium, previous multiple failed surgeries or in the context of preserving superior bulbar conjunctiva for future glaucoma surgeries. Solomon et al reported in a non-comparative study that double-layered AMG combined with the intraoperative injection of triamcinolone significantly reduced the recurrence rates to 3% for primary and 9.5% for recurrent pterygia, a result that is comparable with that after CAG.46

In recurrent pterygia results of AMT has been less favorable in comparison to CAG. A recurrence rate of 37.5% was documented following excision with AMT, which was considerably higher than the 9.1% obtained with CAG.47 AMT and CLAG may be combined in recurrent pterygia. A combined approach including pterygium excision, AMT, CLAG and application of mitomycin C was reported to be beneficial in the management of chronically recurring pterygium in young patients.48

**Shield ulcers of vernal keratoconjunctivitis**
Amniotic membrane transplantation combined with surgical debridement is effective in the management of severe shield ulcers.49 The renewed basement membrane promotes epithelialization, reinforces cellular adhesion and prevents epithelial apoptosis.

**Glaucoma**
AM has been used as an adjunct in glaucoma surgery and also to treat its complications. Its main purpose has been to reduce scarring at the time of filtering surgery, to repair early or late leaks, and act as a cover for valve procedures. Fugishima et al50 used AM to prevent adhesion of the scleral flap to the overlying conjunctiva. The use of AM in late bleb leaks is controversial. Nagai-Kusuhara et al51 reported good long-term results while earlier reports suggested that AMT was not effective in this setting.52

**Conjunctival tumors and OSSN’S**
Excision of conjunctival tumors followed by reconstruction of the ocular surface with AMT has been reported to be successful. The advantages of AMT over conjunctival autografts and mucous membrane grafts in reconstruction of ocular surface include superior postoperative cosmesis, absence of donor site morbidity complicating the harvest of mucosal and conjunctival autografts (CAG) and the ability to clinically monitor local recurrence of tumor beneath the transparent AMG.53

**Lid and orbital surgery**
There are limited reports on the application of AM in oculoplastic procedures. Most have been aimed at reconstruction of the fornices or as a substrate for epithelialisation of conjunctival defects for prosthetic fitting. Poonyathalang et al54 described 10 cases of fornical reconstruction where socket contraction prevented the fitting of a prosthesis. A total of 80% achieved successful fitting following reconstruction. Ti et al evaluated the role of AM in the correction of cicatricial entropion.55 As it promotes rapid epithelialization, it appears to be a promising substitute to conventional grafts like mucous membrane grafts.

**Postoperative management**
A broad-spectrum topical antibiotic is used four times daily till complete epithelialisation occurs. Along with this topical steroids are used for six to eight weeks in tapering doses to reduce surface inflammation.

**Complications of AMT**
There may be early degradation of the membrane and cheese wiring of sutures resulting in loose AM. This may necessitate frequent repeat transplantations. In the immediate postoperative period there may be hematoma formation under the membrane.56 The blood usually absorbs or may need drainage if excessive, which can be done by making a small opening in the graft. Occasionally, a residual subepithelial membrane may persist in some cases and inadvertently opacify the visual axis.

The incidence of post-AMT microbial infections is low (1.6%).57 Gram-positive organisms are the most frequent isolates.58 Sterile hypopyon may occur especially after repeated AMT.59 Rarely calcification can occur in some cases.60

**Conclusion**
AMT has evolved to be an important tool in the surgical armamentarium of the ocular surface surgeon. It has proved to be a viable alternative or option in many challenging clinical situation. The ease of the procedure, repeatability and freedom from intraocular intervention makes it a popular option. The low rate of complications and the avoidance of immunosuppression make it further attractive. With the use of fibrin glue, the surgical technique has become further simplified.

Stringent case selection is needed. The success of AMT is dependent on the underlying condition. The spectrum of clinical indications continues to expand and encompass a varying range of ocular surface pathology.61 The utility of AM in healing ocular surface defects is unquestionable. However, there is still a lack of evidence based on randomized controlled studies to prove the benefits of AMT compared to other alternative modalities of treatment.62
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Introduction

The fungal infections of cornea are unique in many ways. The etiology, presentation, diagnostic tests and their pick up rate, response to treatment and the sequelae all differ from other common bacterial infections. The fungal infections vary in incidence also depending upon the geographic location and the climatic variation. It is important because of its sight threatening nature.1-5 The tropical and the temperate climates show varying predilection to different fungal species.

Keratitis is the most frequent presentation of fungal infection of eye. Fungi are opportunistic in the eye, since they rarely infect healthy, intact ocular tissues unlike certain virulent bacterial species. Ocular fungal infections or ophthalmic mycoses are being increasingly recognized as an important cause of morbidity and blindness more so in tropical countries and developing nations. This is mainly attributed to the fact that the main working population in such countries is involved in the agricultural sector where they are exposed to vegetable matter and other organic contaminants. The varied types of presentation, indolent and chronic nature of the ulcer along with the difficulty in isolating the organism from the specimen have complicated the matter. Injudicious usage of antibiotic / steroid drops, alarmingly increasing trend of self medication and the usage of traditional home made contaminated medication have led to the increasing frequency of the fungal corneal ulcers6.

Fungi are eukaryotic plant-like micro organisms and are one among the five kingdoms of life. There are over 100,000 species of fungi. Since fungi do not have chlorophyll, they absorb food from others. As they don’t use light to make food, they can live in damp and dark places. Opportunistic fungi are harmless commensals which under normal living conditions of tissue seldom produce pathological lesions. Saprophytic fungi grow on dead tissue and are seldom pathogenic. However, under condition of impaired immune defense these can become pathogenic.

The majority of the pathogenic species are classified within the Phyla Zygomycetes, Basidiomycetes, Ascomycetes, or the form group Fungi Imperfecti. Classically, there are two broad groups of fungi: yeasts and moulds. While not mutually exclusive, mould spores germinate to produce the branching filaments known as hyphae. Yeasts, on the other hand, are solitary rounded forms that reproduce by making more rounded forms through the mechanisms as budding or fission. In a growing colony of filamentous fungus, the mycelium can be divided into vegetative mycelium which grows into the medium and aerial mycelium those projects from the surface. All molds of medical importance in corneal disease form septate hyphae.

BROAD GROUPS OF FUNGI PATHOGENIC TO EYE

A. Filamentous fungi
   a. Aspergillus
   b. Fusarium
   c. Curvularia
   d. Paecilomyces
   e. Phialophora

B. Other emerging fungi
   a. Aureobasidium
   b. Rhodotorula
   c. Fonanscea
   d. Pencillium sp

C. Yeast
   a. Candida

Pathogenesis

Ocular infections usually occur as a result of a breach in healthy interaction between the three important deciding factors viz, hosts factor, pathogen and the environment.

While the normal eye has its own defense mechanisms, the eyes with compromised cornea or ocular surface provide the ideal stage for the invasion by the pathogenic organisms. The most common predisposing factors are trauma, foreign body, bullous keratopathy, existing corneal ulceration, herpetic eye disease, severe tear film deficiency and contact lens wear.

The infection can be either exogenous or endogenous. The most common route is external via ocular surface epithelium. One or more components of the flora may take advantage of a situation to penetrate the cornea (an endogenous source
of infection). Alternately, organisms may be inoculated from the external environment at the time of injury (an exogenous source of infection). The virulence of the pathogen, the size of the inoculums and the competence and nature of host defense mechanisms decide the severity of subsequent infection.

The pathogenic mechanisms of fungi include direct physical damage caused by invasion and growth of fungal elements, damage from infiltrating leucocytes and damage produced by fungal toxins and enzymes. The invasion of mycelia usually occurs parallel to collagen lamellae or may be perpendicular with more virulent organisms. This leads to disruption of normal collagen fibre arrangement. The surface mannoprotein adhesins of hyphae or pseudohyphae also inhibit the attachment of neutrophil thus escaping phagocytosis.

Fungal hyphae are large enough to preclude ingestion by neutrophils. However, attempts at phagocytosis results in extra cellular release of lysosomal enzymes and oxygen metabolites. This sets in motion the inflammatory cascade involving plasmin and corneal matrix-derived metalloproteinase with activation of collagenase, proteinase etc which digest the stromal collagen perpetuating the corneal damage.

The fungal cultures of A. flavus and F. solani contain serine proteinase and metalloproteinase activity while Candida albicans strains produce gliotoxin - like metabolite. This can act on a wide-variety of tissue proteins and is thought to contribute to invasiveness of the organism. The status of the host defense mechanisms further, determines the threshold of inoculum at which infection occurs.

In some cases of mycotic keratitis which are responding well to antifungal therapy, a sudden deterioration accompanied by renewed tissue destruction (in the absence of a demonstrable microbial cause) has been noted. This phenomenon is thought to occur because dying fungal hyphae may elicit a type of hypersensitivity reaction.

Administration of corticosteroids can predispose to fungal keratitis by inhibiting chemotaxis thus suppressing ocular immune mechanisms and ingestion by phagocytes. They also block degranulation, and reduce the production of phagocytes.

**Hypopyon ulcer**

In presence of very virulent organisms, the toxin which is secreted by them diffuses in the deeper corneal tissue and into the anterior chamber which ultimately leads to excessive exudation from the limbal as well as iris and ciliary body vessels. These sort of virulent ulcers also have deep and thicker infiltrate. The exudation in the anterior chamber is not sterile as in most cases of bacterial infection. In cases of fungal infection the anterior chamber exudation also may contain the hyphal elements due to the penetration of the stroma by the invasive hyphae. This is more common than in bacterial ulcers due to this invasive nature of most filamentous fungal hyphae.

**Specific fungal pathogens**

1. **Aspergillus**\(^{8-10}\): Aspergillus is a filamentous and ubiquitous fungus commonly isolated from soil, plant debris, and indoor air environment. Among these, Aspergillus fumigatus is the most commonly isolated species, followed by Aspergillus flavus and Aspergillus niger. Aspergillus spp. are well-known to play a role in three different clinical settings in man: (i) opportunistic infections; (ii) allergic states; and (iii) toxicoses. Immunosuppression is the major factor predisposing to development of opportunistic infections. Since Aspergillus spp. are found in nature, they are also common laboratory contaminants.

2. **Fusarium**\(^{11-12}\): Fusarium is a filamentous fungus widely distributed on plants, in soil and found in normal mycoflora of commodities, such as rice, bean, soyabean, and other crops. While most species are more common at tropical and subtropical areas, some inhabit in soil in cold climates. Fusarium is one of the emerging causes of opportunistic mycoses. The most virulent Fusarium spp. is Fusarium solani. Fusarium spp. produces mycotoxins.

3. **Dematiaceous Fungi**\(^{13}\): These saprophytic fungi are distinguished by the brown pigmentation of their colonies. A number of their members including Curvularia, Alternaria, and Cladosporum have been reported as opportunistic pathogens.

   a) **Curvularia**\(^{14}\) is a dematiaceous filamentous fungus. Curvularia lunata is the most commonly encountered species. Importantly, the infections may develop in patients with intact immune system. However, similar to several other fungal genera, Curvularia has recently emerged also as an opportunistic pathogen that infects immunocompromised individuals.

   b) **Alternaria**\(^{15-17}\) is the most common fungus of this group and is isolated from human infections. The species have emerged as opportunistic pathogens particularly in patients with immunosuppression, such as the bone marrow transplant patients.

   c) **Aureobasidium pullulans**\(^{18}\), an emerging pathogen, which comes under phaeohyphomycoses, is a dematiaceous fungus. Though the pathogenicity of phaeohyphomycosis was questioned towards corneal disease, the reports are
increasing. One needs to be careful in both clinical and microbiological evaluation for this organism as it has certain clinical characteristics and require specific microbiological evaluation. The ulcers are usually central in location with multiple round ball like infiltrates around the ulcer with extension to periphery.

4. Yeasts: The majority of yeast infections are due to various Candida species predominately C.albicans. The candida keratitis is more frequently encountered in temperate climates while it is a rare entity in tropical climate.

5. Unusual fungal pathogens: Case reports are available in literature about keratitis involving unusual fungal pathogens like Scedosporium, Phialophora, Metarrhizium anisopliae etc.

Clinical presentation

The clinical features of fungal keratitis are non-specific and may be confused with indolent ulcer of viral and bacterial origin. The distinctive features of the fungal ulcer are as under:-

a. Hyphate ulcer: Fungal ulcer has a dry appearing epithelial surface with a rough texture and dirty grey-white colour (fig 1). The epithelium may be elevated and intact or occasionally it may ulcerate. It has delicate feathery branching hyphae with surrounding stromal infiltrate. The extension of the hyphate margins beyond the ulcer edge present a distinctive feature and is a useful diagnostic finding.

b. Severe ocular reaction: The typical fungal keratitis produces violent ocular reaction. There is appreciable ciliary flush and flare in anterior chamber (fig 1).

c. Hypopyon: Hypopyon is invariably present in fungal keratitis and usually result from sterile reaction to fungus and its toxins. However, fungi may invade the anterior chamber through intact Descemet's membrane and result in a fixed hypopyon (fig 1).

d. Satellite lesions: Satellite lesions are discrete stromal infiltrates that surround the ulcer and are separated by clear cornea (fig 1).

e. Pigmented Ulcers: The ulcer infiltration can be pigmented (eg: brown) in infection due to dematiaceous fungi(fig 1).

f. Endothelial plaque

An endothelial plaque is composed of fibrin and leucocytes. It is located under the stromal lesion and may be present in the absence of hypopyon. Micro abscesses, satellite lesion and ring infiltrates are non-specific and represent an immune response.

In advanced cases, the entire cornea becomes homogenously yellowish-white and can resemble any microbial keratitis. Stromal ulceration and necrosis may lead to perforation and endophthalmitis. This is especially a threat with Fusarium solani keratitis in association with inappropriate use of topical corticosteroids.

Yeast keratitis causes a small oval ulceration with an expanding, discrete, sharply demarcated, dense, yellowish-white stromal suppuration lacking delicate features of filamentous organisms.

Many patients receive some sort of treatment before presenting to the ophthalmologist for expert opinion which may alter the morphology of the ulcer causing more confusion to the etiological diagnosis. Thorough examination of lid margin and both bulbar and palpebral conjunctiva of the ipsilateral eye is essential to find out any offending object. Initial measurement of size of epithelial defect along with infiltration should be carried out and documented with proper color coding. This will guide the clinicians for monitoring the lesion. Limbal/scleral extension should be found out to modify the standard therapy. Posterior segment evaluation is indicated if there is suspicion of endophthalmitis.

Laboratory diagnosis:

A microbiological work up of a suspected infectious ulcer must be done before the start of antibiotic treatment. Corneal scraping provides material for microbiological diagnosis, debrides necrotic tissues and enhances antibiotic penetration.

The commonly used techniques for identification of etiological agents;

1. Direct microscopic examination
2. Culture

Method of sample collection:

Corneal scraping:-

After getting informed consent from the patients and proper explanation of the procedure, the affected eye should be anaesthetised with 0.5% proparacaine eye drops. All sterile surgical precautions should be taken to avoid contamination while sample is collected. After application of a Barraquer wire speculum, the superficial debris and mucus strands are to be cleaned and the ulcer should be scraped from the base and the leading edge with blunt tipped iris repositor. Care should be taken not to perforate by avoiding thinned necrotic areas and the direction should be always towards one side rather than making to and fro movements. Also cases with
intact epithelium and deep abscess with comparatively less infiltrate superficially should be scraped in depth with due care to prevent perforation.

**Anterior chamber (AC) paracentesis**: 
In very rare circumstances like suspected fungal infection with repeated negative culture reports but progressive infection, AC paracentesis is indicated. It is also called for, when there is scanty material available from scraping and there is thick hypopyon. Sharp 22/20 gauge needle is inserted into the anterior chamber between 6 O’clock and 7 O’clock area directly into the hypopyon and the material is aspirated. The procedure must be carried out under aseptic precautions like routine intraocular surgery.

**Corneal biopsy**: 
It is indicated in cases with deep stromal abscess or in case where repeated culture shows negative reports but there is strong suspicion of infection. The cornea is anaesthetized and 0.2-0.3mm trephine is used to outline the area to be biopsied. Usually a depth of about 0.1-0.2 mm is dissected out. The tissue is then sent for histopathological as well microbiological analysis.

Post LASIK cases: Fungal infections after LASIK, though rare, are reported in literature. Sample collection from corneal infection after LASIK surgery requires special precaution. Scraping of the surface is not indicated in these eyes due to fear of button holing of the flap. It can be performed by lifting of the flap, as following infection the flap becomes edematous and thus provide little resistance. Therefore, careful handling of the flap is mandatory. The specimen collection should be done both from the bed and undersurface of the flap. If there is necrosis of the flap, either excision or amputation of the flap should be done to reduce the load of infection.

**Transportation and processing of collected material**: 
The collected sample should then be transferred to cotton tipped applicator from the tip of repositor and dipped into the bacterial culture tube and the freshly prepared Sabouraud’s dextrose agar( the fungal culture media). The final part of the sample collected should be used for preparing slides for the KOH wet mount and Gram’s staining. The commonly used staining techniques employed are:

a) Gram’s staining  
b) Wet KOH (10%) mount  
Other staining techniques used in detecting fungus include:

a) Geimsa staining  
b) Gomori Methenamine Silver (GMS)  
c) Periodic acid-Schiff (PAS)  
d) Calcoflour white  
e) Acridine orange

Identification of the fungal genus by direct examination is generally not considered possible. Studies have suggested that calcium alginate swabs yielded significantly greater growth than blade in mycotic ulcers though not significant for bacteria and mixed ulcers.

The potassium hydroxide (KOH) wet mount and its modifications like Ink KOH are widely used for the rapid detection of fungal hyphae. The ink in the ink KOH technique gives a good contrast which helps the examiner in detecting the hyphae from otherwise colorless background.

The Giemsa stain, Gomori methenamine silver (GMS) and the periodic acid-Schiff (PAS) stains are special stains for detection of fungi in tissue. Various studies have reported varying sensitivities for these stains. In recent years, nonspecific fluorochromatic stains like Calcoflour white is found to be more sensitive than KOH wet mounts in detecting the common ocular fungi such as *F. solani* and *A. fumigatus* in corneal scrapes. Acridine orange stain has also been found useful to detect fungal hyphae in corneal scrapes in recent studies. Fluorescein-conjugated concanavalin A was found to provide consistently bright staining of the fungal structures in corneal scrapes and is thought to be a promising first-line fluorochromatic stain to visualize fungi in ocular samples.

**Culture technique**: 
Culture is the ‘gold standard’ technique of investigation in microbial keratitis. The specimen collected from the corneal scraping should be cultured for bacteria and fungi regularly. This should be done routinely in case of repeat cultures where the clinical course is progressive in spite of logical treatment. The specimen for the culture and sensitivity should be ideally taken before any sort of antibacterial / anti fungal medication is started on as this may affect the positivity. If the patient is already on medication, it is recommended to even stop the treatment for 24-48hrs under strict supervision of the clinician before culturing to enhance the chance of positive growth. Though liquid medium is highly sensitive for demonstration of pathogen, they are less specific than solid media because quantification is lacking in former.

**The commonly used culture media**: 

a. Sabouraud’s dextrose agar incubated at 25°C,
b. Brain heart infusion broth incubated at 25°C,
c. Thioglycolate broth

Composition of Sabouraud’s media commonly consists of glucose 20g, peptone 10g, agar 15g, water 1L which is steamed to dissolve and pH adjusted to 5.4. It is then autoclaved at 1150°C for 15mts with added gentamicin and chloramphenicol. Antibacterial antibiotics, such as chloramphenicol or a penicillin-streptomycin combination, are usually incorporated in fungal culture media to suppress bacterial growth and permit the isolation of fungi alone. But chemicals like cycloheximide suppress the growth of fungus. The fungal culture tubes are stored at 25o C in biological oxygen demand (BOD) incubator and taken every third day for observing growth. If any growth is noted, the LactoPhenol Cotton Blue (LPCB) staining is done to study the detailed morphology of the species.

**Growth of organisms in culture medium:**

Almost all majorities of fungi (filamentous) can grow within 3 days, but it is not unusual for them to take 5-7 days to grow and upto one-fourth may take upto14 days. Therefore, culture plates should be kept for 3 weeks time for ocular fungi isolation.

**Alternative emerging investigations:**

1. **Molecular techniques – PCR**

Polymerase chain reaction is being more commonly used nowadays in diagnosing microbial keratitis because of its rapid results and ability to pick up cases in even partially treated cases 35. The advantage is that it requires only a very small material for analysis. It can detect even non viable organism the significance of which lies in pre treated cases where superficial scraping will provide only non viable organism which will invariably give culture report as negative. PCR usually targets 28S rRNA sequence which is common to most of the pathogenic fungi. Sujith Nayanar et al in their prospective evaluation of PCR in cases of presumed fungal keratitis showed 50% positivity on PCR whereas only 25% were positive on culture 35.

2. **Clinical aids like Confocal microscopy** is also being tested and tried for identifying fungal keratitis whereby actual fungal elements can be visualized in vivo 36. Various studies have come out showing the efficacy of confocal microscopy in detecting organisms. It provides epithelial, stromal, endothelial details and makes it possible to observe microorganisms in vivo without use of dyes, stains or tissue fixation. In cases of fungal keratitis with deep seated infiltrates and delayed growth in culture, confocal microscopy can detect fungal filaments accurately and thus preclude the need for more invasive procedures like corneal biopsy.

**Initiating treatment:**

The general guidelines of treatment for fungal keratitis are same as those for most of the other infective keratitis, but the duration is usually much longer 37. Antifungal treatment is usually not started as an empirical therapy unless very strongly suggested by the clinical appearance of the ulcer or mode of injury. Antifungal should be instituted at the earliest following the availability of the smear report. The culture report, as mentioned previously takes time to give conclusive evidence. Therefore, presence of hyphae in wet KOH mount or Gram’s smear is enough evidence to start on antifungal medication. The newly introduced faster molecular techniques using PCR may also prove to be beneficial in providing an early report.

The common class of agents used as anti-fungals include:

- Polyene antibiotics
- Imidazoles
- Triazoles
- Pyrimidines
- Nystatin
- Echinocandins

The treatment for fungal keratitis is complicated mainly by factors namely; deep stromal infiltration which is common in mycotic keratitis and poor ocular penetration by the anti fungal agents.

Polyene antibiotics 38 interact with cell membrane sterols primarily ergosterol, which causes increased permeability and leads to cell lysis. The two main agents in this group are Amphotericin B and Natamycin . 5% topical suspension of Natamycin is the most common polyene agent that is used in fungal keratitis. Its disadvantage includes limited penetration into deeper layers of cornea and the need for frequent dosing. Epithelial debridement is found to be helpful in achieving deeper penetration of the medicine. Amphotericin B is another polyene which is available in 0.15% topical suspension and also in systemic form as tablets. As with Natamycin, drug penetration is a problem in Amphotericin B also and higher concentrations are toxic to ocular surface. Subconjunctival, intracorneal or intracameral administration is also possible with this agent. The toxicity of the agent especially corneal and ocular surface toxicity limits its widespread use in fungal keratitis.

Azoles: Imidazole and triazoles comes under this group which inhibit the biosynthesis of ergosterols of fungal cell wall and
also some unrelated direct inhibition of cell wall synthesis.39
Imidazoles include Econazole, Miconazole and Ketoconazole.
The triazoles include Fluconazole, Itraconazole and the
newer agents like Voriconazole and Posaconazole. Triazoles
have broader spectrum, longer half life and fewer side effects
than imidazoles40.

Pyrimidines are group of anti metabolites with known
antifungal activity. Flucytosine is a fluorinated pyrimidine
and is the main agent in this group.

Voriconazole41,42 is a newer triazole, which is structurally
related to fluconazole which acts by inhibiting cytochrome
P450 14 α demethylase. This is fungistatic to Candida but
fungalidal to Aspergillus. It also has broad spectrum of activity
that includes Fusarium sp. It is being tried intracorneal in
many difficult cases with encouraging results as reported in
literature. Posaconazole43 is also a new triazole antifungal
agent, with few available reports in literature showing utility
in difficult cases of fungal keratitis.

Newer antifungals with different mechanisms of actions have
been introduced recently. The glucan synthesis inhibitors are
agents which are presumed to block fungal cell wall synthesis
(rather than cell membrane) by inhibiting the enzyme 1,3-β
glucan synthase. There are three such agents at present with
all three belonging to the chemical family also known as the
Echinocandins. The agents are Caspofungin, Micafungin and
Anidulafungin. Their spectrum of activity is mainly against
Candida and Aspergillus sp. with limited activity against
Fusarium sp.

As per the currently accepted practice patterns Natamycin
5% topical suspension is considered as first line therapy for
filamentous fungal keratitis37. Depending upon the load
of infection, the dosage should be as frequently as 1 hourly
which has to be slowly tapered over many weeks depending
upon the clinical improvement. The Natamycin topical
suspension has to be shaken well before usage to utilise
the medicine to the maximum. Natamycin is specifically
effective against Fusarium sp. The second line agent is
Amphotericin B which may be considered as the first line if it
is a yeast infection. Amphotericin B is also recommended for
filamentous keratitis caused by Aspergillus Sp. 5-FC is often
added to Amphotericin B to give additive effects

The role of Azoles especially Triazoles is mainly as adjunctive
to primary therapy of polyene. Oral antifungal agents may
be used in fulminant and progressive cases as adjunctive to
topical medication. It is also recommended in deep keratitis,
associated scleritis and endophthalmitis. It is also part of
treatment following penetrating keratoplasty for therapeutic
purpose in a case of deep fungal keratitis. The usually used
agents are Ketoconazole (200-600mg/day) for filamentous
fungal keratitis and Flucanazole (200-400mg/day) for yeast
and Itraconazole (200mg/day) for severe yeast keratitis.
Itaconazole also shows activity against Aspergillus but lesser
towards Fusarium species.

The role of newer agents like Voriconazole is in severe Keratitis
not responding to the above agents. It has got excellent
penetration after topical and oral administration. The current
limiting factor for this broad spectrum agent is the cost
involved in it, the need for preparing topical medicine from
injection and short shelf life of the medicine. Because of this
limitation in our setup this is usually reserved for difficult
cases non responsive to routine agents. Echinocandins are
very potent agents against candida species.

Intracameral and intracorneal/Intrastromal administration
may be considered in some cases with intraocular extension
or anterior chamber involvement. (Amphotericin B 5-10
µgm /0.1ml)44 and voriconazole (100 µg/0.1ml) 41-42are
the agents preferred for intracameral usage. Amphotericin B
carries the risk of endothelial toxicity. Voriconazole has a shelf
life of 24-48hrs after preparation. Elimination of voriconazole
after intracameral injection exhibited an exponential decay
with a half-life of 22 min. Depending upon the response to
treatment the injection may have to be repeated multiple
times.

Resistance to anti fungals is rarely seen except for flucytosine
for which it is reported. Topical anti fungals may have to be
used for prolonged duration. At least 6weeks to 3months of
treatment may be needed depending upon the extent of
involvement. The frequency of medication can be decreased
by 10 days to 2 weeks if clinical improvement is noticed.

Supportive therapy:
The specific antifungal treatment should be supported by:

a) Strong Cycloplegic (preferable atropine eye ointment)
b) Lubricating eye drops/gel formulation
c) Anti-inflammatory drops (NSAID)preferably bromfenac
d) Antibiotic eye drops(Ofloxacin/Moxifloxacin/Gatifloxacin)
  -- to take care of possible superadded bacterial keratitis.
e) Systemic pain killers (SOS)
f) Systemic Doxycycline (may be added for its Matrix
  Metalloproteinase(MMP) inhibitory action)

The cocktail treatment of maximum antibiotic, antifungal
(multiple agents) and antiviral should be strongly discouraged
as this will do more harm than good to the patient.

Monitoring the progress & Assessing the healing:
The fungal corneal ulcers are notorious for their chronic nature and slow response to treatment. The most important part of the treatment is daily or alternate daily reassessment of:

1. Symptomatic improvement
2. Systemic control of risk factors like diabetes
3. Epithelial healing
4. Amount & depth of infiltration
5. Appearance of fresh infiltrates
6. Status of Hypopyon
7. Involvement of sclera or vitreous spread

General pattern of improvement shows following pattern:

Symptomatic improvement
↓
No fresh infiltrates or satellite lesion
↓
Decrease in hypopyon (total disappearance may take longer time than bacterial)
↓
No increase in size of ulcer
↓
Consolidation of margins of infiltration
↓
Epithelial healing and scarring

It should be noted that epithelial healing can be very slow and in spite of resolution of other signs usually the epithelial defect can persist for longer time especially in diabetic patients. Non healing epithelial defect alone should not be taken as a sign of non responding fungal keratitis. This needs a careful observation by preferable same clinician on regular interval to avoid misinterpretation and un necessary alteration or premature termination of an otherwise successful treatment.

Role of anti-inflammatory drops/ corticosteroids:

Even in a keratitis that is responding to the treatment very well, the unusually severe inflammation can be deleterious to the cornea. This is not very uncommon. The eye may be angry looking, corneal tissue necrosis and severe thinning may be noticed which can lead to impending perforation or a perforated corneal ulcer. After an initial sign of resolution of hypopyon sudden resurgence in hypopyon without corresponding increase in corneal ulcer size or infiltration is another presentation of inflammation taking over the infective component. The clinician has to be very astute in such occasion to select proper alteration in the existing treatment. In most cases the inflammatory component can be controlled in the initial part of the treatment with Topical NSAID drops. Use of topical steroids in uncontrolled fungal keratitis is absolutely contraindicated. Systemic steroids are an option which can be used in extreme conditions where topical medicine alone is not doing enough help. This may not be ideal in diabetic patients who are more prone to fungal keratitis. In the latter half of treatment where the infiltration is more or less consolidated fully and scarring has started and the patient has been on antifungal drops for >3weeks topical steroids preferably fluoromethalone or loteprednol may be used under very strict monitoring. An efficient management of inflammatory component is necessary in achieving a successful outcome.

Surgical options in active phase 44-49

It is always better to treat an active fungal keratitis in a conservative manner. Surgical management in an active ulcer poses lot of difficulty which even carries risk of incomplete removal of the infective load, inadvertent spread to vitreous, recurrence of keratitis in the donor cornea and secondary glaucoma that can be intractable to medical management. Approximately 15-30% of patients require surgical intervention. There are situations in which a therapeutic keratoplasty may have to be considered in early phase itself. These include;

a) Non responding progressive ulcer
b) Large ulcer/deep abscess
c) Limbus involved /threatened to be involved with risk of scleritis (fig 4)
d) Badly perforated/impending perforation (fig 5)
e) Associated signs of endophthalmitis

The surgical treatment of choice is therapeutic keratoplasty in such a way that the involved corneal tissue must be removed fully with a possible 0.5mm-1mm clear margin45. This avoids the risk of re infection of graft to reasonable extent. The main goals of surgery are to control the infection and to maintain the integrity of the globe. Topical antifungal therapy, in addition to systemic fluconazole or ketoconazole, should be continued following penetrating keratoplasty. The use of topical corticosteroids in the postoperative period remains controversial. Maximum attempt must be done to preserve the lens and/ or posterior capsule. Lens replacement even in cataractous lens should be deferred to be done along with
Optical procedure later once the infective component is totally removed. It is also critical to thoroughly irrigate the anterior chamber with antifungal medication (preferably Voriconazole). For ulcers larger than 8 mm diameter or if the ulcer is near the limbus, it is very important to open the Conjunctiva and retract it. This allowed us to determine if the infection had invaded the sclera necessitating additional surgical intervention.

POSTOPERATIVE MANAGEMENT

Recurrent fungal infection can be particularly difficult to treat. Following PKP both systemic and topical antifungal agents must be used. Corticosteroids were not used unless significant inflammation was present. Chances of graft failure are high due to:

a) Re-infection
b) Graft rejection due to non-usage of steroids and large grafts
c) Primary graft failure due to inflammation
d) Secondary glaucoma sometimes intractable
e) Associated endophthalmitis and associated complications.

In cases of small perforations (<2mm) or descemetocele, cyanoacrylate glue may be used to seal the perforation which will help in reforming the AC and avoid a need for therapeutic keratoplasty. In small peripheral lesions a conjunctival hooding/ flap can be considered as a simple alternative technique to tide over the acute situation.

In worst scenario with severe perforation with scleritis and endophthalmitis in immune compromised patient or uncontrolled diabetic patient decision to eviscerate the eye may have to be taken to avoid further spread of the infection and to avoid a situation of painful blind eye.

Role of C3R:

Role of collagen cross linking in halting progression in infectious keratitis is being studied. Few reports (oral and poster publications) have come stating its success in Fusarium Keratitis. Martins S A et al have found no effect of cross linking in their experimental study on Candida albicans culture growth.

No standardised recommendations are currently available in this regard.

Visual rehabilitation: In an eye that survives the infection without perforation and healed with a scar or in an eye that had to undergo therapeutic keratoplasty and survived up to a reasonable time period of 6 months to 1 year without rejection, re-infection and secondary glaucoma may undergo optical keratoplasty to recover vision. Additional procedures like cataract extraction and IOL implantation can be planned more safely with such procedures to help in better visual rehabilitation. Poor visual prognosis is usually associated with perforated ulcers, those associated with scleritis and endophthalmitis and those have intractable glaucoma after keratoplasty.
Fig 1: Classical clinical features of a presumed fungal corneal ulcer: Feathery margins, satellite lesion, hypopyon, dry & rough texture, brown pigmentation (dematacious fungi), ‘angry looking’ conjunctival congestion.

Fig 2: Progressive fungal keratitis with superior limbal involvement.

Fig 3: Healing ulcer with descemetocele and impending perforation.

Fig 4: Perforated ulcer with vascularised pseudocornea and anterior staphyloma.

Progressive fungal ulcer

Operated therapeutic keratoplasty which cleared the infective period

Endothelial and stromal rejection in early postoperative period.

Sujith Nayanar et al - Fungal Keratitis
<table>
<thead>
<tr>
<th>Antifungal group</th>
<th>Mechanism of action</th>
<th>Main Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyene antibiotics</td>
<td>Interact with cell membrane sterols primarily ergosterol. Cause increased permeability of cell membrane.</td>
<td><strong>Agents/route/conc.</strong> Amphotericin B 0.15%-0.3%  Natamycin 5%</td>
</tr>
<tr>
<td>Imidazoles</td>
<td>Inhibit ergosterol synthesis by inhibition of cytochrome P450 dependent 14 demethylase.</td>
<td><strong>Agents/route/conc.</strong> Clotrimazole, 1% topical Miconazole topical 1% drops, 2% cream, subconjunctival Ketaconazole Topical 5% Oral 200-400mg/d</td>
</tr>
<tr>
<td>Triazoles</td>
<td>Inhibit ergosterol synthesis by inhibition of cytochrome P450 dependent 14alpha demethylase.</td>
<td><strong>Agents/route/conc.</strong> Fluconazole 0.2% topical, Oral 200mg/day Itrakcanazole 1% topical, Oral 200mg/day Voriconazole 1% topical, Oral 200-400mg/d</td>
</tr>
<tr>
<td>Pyrimidines</td>
<td>Antimetabolite-blocks fungal thymidine synthesis.</td>
<td><strong>Agents/route/conc.</strong> Fluycytosine 2% topical</td>
</tr>
<tr>
<td>Echinocandins</td>
<td>Glucan synthesis inhibitors.</td>
<td><strong>Agents/route/conc.</strong> Capsofungin, Micafungin Anidulafungin</td>
</tr>
</tbody>
</table>
Reference


Dr Sujith Nayanar did his MD and then his corneal Senior residency from Dr RP Cente, AIIMS. He then took DNB and FRCS and did a corneal fellowship from the university of Helsinki and is now working as consultant in Cataract and Corneal surgery at Vasan eye hospital, Kottayam.
Anti VEGF in Retinal Diseases – An Evidence Based Review

INTRODUCTION

VEGF (Vascular endothelial growth factor) is one of the major regulators among the angiogenic factors studied so far. VEGF plays a central role in the development of new vessels as in CNV and in PDR. Anti-VEGF therapy, introduced to ophthalmology less than a decade ago, has fast become a mainstay of managing diseases such as age-related macular degeneration (ARMD), Clinically significant macular edema (CSME), Branch / Central retinal vein occlusions (BRVO/CRVO), Proliferative diabetic retinopathy (PDR) and it may expand to even more indications.

VEGF

The VEGF family includes placenta growth factor, VEGF-A, VEGF-B, VEGF-C, VEGF-D and VEGF-E. Briefly, VEGF-A plays a pivotal role in the development of pathologic angiogenesis in ischemic and inflammatory diseases. VEGF is a 35- to 45-kd homodimeric protein originally isolated as a vasopermeability factor and later cloned and identified as an angiogenesis factor. By alternative exon splicing of a single gene consisting of eight exons, several VEGF isoforms can be generated [1]. Up to six different VEGF isoforms are derived through alternative splicing of messenger RNA (mRNA). VEGF165 appears to be the isoform most responsible for pathologic ocular neovascularization in humans. VEGF165 is the most abundantly expressed VEGF isoform, and has the optimal characteristics of bioavailability combined with high biological potency. VEGF165 possesses a combination of properties as that of short isoforms of VEGF like its diffusible nature, a significant fraction remains bound to the cell surface and the extracellular matrix as the larger VEGF isoforms.

Hypoxia is a major regulator of VEGF expression which distinguishes VEGF from other growth factors that have been postulated to have a role in ocular neovascular diseases, including insulin-like growth factor-1, fibroblast growth factors (FGF), epidermal growth factor and placenta growth factor. Many cells in the eye produce VEGF and within the retina, these include RPE, pericytes, endothelial cells, glial cells, muller cells and ganglion cells. In the human eye, elevated vitreous and aqueous VEGF levels strongly correlate with retinal ischemia-associated neovascularization in conditions like diabetic retinopathy, retinal vein occlusion and retinopathy of prematurity [2],[9].

Study conducted by Ishida et al in mouse model of ocular neovascularisation showed that both the absolute and relative levels of VEGF 164 (the equivalent of human VEGF 165) increased to a greater degree during pathological neovascularisation than during physiological neovascularisation.

Physiological actions of VEGF

VEGF is crucial for embryonic and early postnatal vasculogenesis and angiogenesis.[1]. In adults it is a potent vasodilator and increases microvascular permeability. It has a role in cardiac muscle remodelling and skeletal muscle regeneration and endochondral bone formation. Glomerulogenesis and renal glomerular capillary function of the kidneys are totally dependent on VEGF. It also plays an important role in female reproductive cycle.

VEGF inhibitors or Anti VEGF

Anti-VEGF aptamers are stable small RNA-like molecules that bind exclusively and with high affinity to the 165-kDa isoform of human VEGF. Pegaptanib sodium, an oligonucleotide known as an aptamer, binds and inhibits only the extracellular isoforms of VEGF that are at least 165 amino acids in length. [3] Multiple biologically active forms of VEGF-A are generated by both alternative mRNA splicing and posttranslational modification (proteolytic cleavage), [4,5] and two of these forms (VEGF165 and VEGF121) have been detected in choroidal neovascular lesions. Pegaptanib (Macugen, Eyetech Pharmaceuticals/Pfizer) is a PEGylated (i.e. conjugated to polyethylene glycol) neutralising RNA aptamer with an extremely high affinity for human VEGF165. [6] An important feature of aptamers is that, unlike a number of recombinant human proteins, they are essentially non-immunogenic. Intravitreal administration of pegaptanib has been shown to significantly inhibit fluorescent, pathological retinal neovascularisation and VEGF-mediated vascular leakage in rodents [7, 8]. Pegaptanib was approved by the US Food and Drug Administration (FDA) for the treatment of exudative AMD in December 2004.

In contrast to Pegaptanib, Bevacizumab (Avastin; Genentech, South San Francisco) a full-length, humanized monoclonal antibody against VEGF and Ranibizumab (Lucentis;
Role of VEGF and use of Anti VEGF in AMD

The role of VEGF as a critical factor in the control of the growth of abnormal blood vessels from the choroid directly attacks a central problem in this disease. The profound vascular permeability induced by VEGF is potentially of even greater importance in the treatment of established neovascular AMD lesions, in which leakage of fluid from new vessels causes visual loss through retinal edema and exudation, subretinal fluid and hemorrhage. [2]

The importance of VEGF as a therapeutic target derives from its roles in two of the most basic processes within a typical lesion of advanced AMD: neovascularization and vascular leakage. The neovascular form is responsible for 80 to 90% of cases of severe vision loss due to ARMD. Given the increasing prevalence of neovascular ARMD and the burden of associated vision loss, it is important to define treatment benefits that are meaningful to the patient. Neovascular AMD often has a poor prognosis, resulting in a rapid and progressive loss of visual acuity and contrast sensitivity. Such losses have a profound effect on patients' quality of life and their ability to perform everyday tasks. Photodynamic therapy (PDT), currently the most thoroughly investigated definitive therapy, is useful mainly for the classic types of neovascular AMD. However, most angiographic lesions of patients who undergo fluorescein angiography for neovascular AMD are subfoveal and occult; only 20% of subfoveal lesions are predominantly classic. Other treatment options such as submacular surgery and steroid-based therapies appear less favorable on current evidence. The strong supportive evidence from animal studies defined VEGF as an optimal therapeutic target. It is hoped that by using a more selective and less destructive approach, vision loss induced by the treatment itself might be reduced. VEGF over expression induces endothelial cell proliferation and increases vascular permeability, properties that can be detected clinically as the presence of subretinal fluid and hemorrhage. [2]

In the VEGF Inhibition Study in Ocular Neovascularisation (VISION) trial, patients with subfoveal CNV were randomised to receive intravitreal injections of Pegaptanib in three different doses or placebo; the placebo group could use PDT in classic lesions. [19] The group which had 0.3 mg Pegaptanib, vision loss was prevented.70% with pegaptanib Vs 55%with placebo lost least than 3 lines in visual acuity (P<0.001). Angiographic assessment showed treated group had slower CNV lesion growth and lower CNV size and leakage by 30 and 54 weeks. The trial also demonstrated a good safety profile with no antibodies detected against pegaptanib sodium and very low rates of endophthalmitis.

In a study done by GonzalesCR, treatment of two groups of early lesions of AMD with intra vitreal Pegaptanib was done...
which showed benefits with pegaptanib treated eyes than in usual care eyes. However with the advent of newer anti VEGF agents, the use of Pegaptanib in treating AMD has come down.

Bevacizumab is an anti VEGF agent, which has an off-label indication for treatment of CNV in AMD patients. In an open-label Systemic Avastin for Neovascular AMD [SANA] trial, exudative AMD patients who were not candidates for PDT received intravenous Bevacizumab [20]. Due to its side effects, patients were excluded if they had severe systemic hypertension or were on anticoagulation therapy or had thromboembolic events or proteinuria. Although there was improvement in visual acuity, due to its side effects, local drug delivery by intravitreal route was given to treat CNV in AMD, though it is an off-label indication of the drug [21]. Rapid improvement in visual acuity and reduction in retinal thickness have led to the widespread use of bevacizumab in the treatment of subfoveal CNV in AMD patients.

Bevacizumab is more commonly used than ranibizumab as it has comparable outcomes but is much cheaper [22]. The second-year CATT [Comparison of age-related Macular Degeneration Treatment Trials] reports 2012 also indicate that bevacizumab (Avastin®) is equivalent to ranibizumab (Lucentis®) in the treatment of wet AMD through two years when using similar dosing regimens. The study also showed that monthly dosing produced slightly more vision gain than an as-needed regimen. The final visual results, however, were similar in all treatment groups, regardless of dosing frequency, with 60 percent or more of the patients achieving driving vision (20/40 vision or better).

Ranibizumab binds to and inhibits the biologic activity of all forms of VEGF-A and their active degradation products. The Minimally Classic/Occult Trial of the Anti-VEGF Antibody Ranibizumab in the Treatment of Neovascular AMD [MARINA] demonstrated that 95% of ranibizumab treated patients with minimally classic or occult CNV experienced improvement or stabilization of visual acuity compared to 62% of sham treated patients after 12 months. More important, almost 40% of ranibizumab treated patients had an improvement in vision of 15 letters compared with sham treated patients.

The Anti-VEGF Antibody for the Treatment of Predominantly Classic Choroidal Neovascularization in AMD [ANCHOR] study, a randomised, double masked, sham controlled clinical trial of patients with predominantly classic CNV of AMD treated with ranibizumab and sham verteporfin PDT or sham injection and verteporfin PDT, reported that approximately 95% of the ranibizumab treated patients maintained or improved vision compared to 64% of patients treated with PDT after 12 months. It also reported that after 24 months of treatment, 90% of ranibizumab treated patients lost less than 15 letters in visual acuity compared to 65.7% of patients treated with PDT. Almost 80% maintained or improved vision after 24 months with ranibizumab.

The MARINA and ANCHOR studies evaluated monthly ranibizumab dosing. The phase IIb randomised, double blind, sham injection controlled study of the efficacy and safety of ranibizumab administered monthly for 3 months and then quarterly in patients with subfoveal CNV secondary to AMD [PIER]. Similar to the MARINA and ANCHOR study results, the mean change in visual acuity from baseline improved over the first three months; however the treatment effect declined on quarterly dosing of ranibizumab. Rosenfeld performed a trial with 3 consecutive monthly injection of ranibizumab (0.5 mg) and performed only if there was loss of 5 letters in conjunct with fluid at macula in OCT, new macular haemorrhage, new onset classic CNV, or persistent macular edema detected by OCT at least 1 month after previous injection of ranibizumab. With OCT guided therapy, the mean visual acuity improved by 9.3 letters and mean OCT central retinal thickness decreased by 178 µm in 40 patients in the study. The mean injection free interval was 4.5 months before another injection was necessary [50].

Several multi-centre studies are investigating the benefit of combining PDT with intravitreal anti-VEGF [23, 24]. As neovascularisation matures, anti-VEGF agents become less effective and therefore will have a limited effect on the more established vasculature observed in late stages of exudative AMD [25, 26]. Studies have demonstrated that PDT indirectly upregulates VEGF which may even stimulate CNV growth [27, 28]. So a combination of Anti-VEGF with PDT breaks this vicious cycle.

The two year phase I/II RhuFab V2 Ocular Treatment Combining the Use of Visudyne to Evaluate Safety [FOCUS] study [29] indicated that Ranibizumab in combination with PDT was more effective than PDT alone in maintaining and improving vision. A large, retrospective, internet-based registry study examined patients with CNV due to AMD who received one or more combination treatments with 1.25 mg of Bevacizumab within 14 days of PDT [30]. The combination therapy with Bevacizumab and PDT had led to visual benefits in most patients and the number of retreatments was very less than with monotherapy alone.

**Retinal ischemic retinal disorders.**

These disorders include retinopathy of prematurity, diabetic retinopathy, retinal vein occlusion, and others, together accounting for the majority of new-onset legal blindness.
each year. Vascular endothelial growth factor has been shown to possess many properties which suggest that it may mediate the majority of intracocular neovascularization associated with ischemic retinal disorders. Vascular endothelial growth factor (VEGF) was originally described in highly vascularized tumors where its expression is increased by hypoxia. VEGF is an endothelial mitogen, angiogenic protein, and potent vasopermeability factor that mediates its effects through the endothelial cell-specific, high-affinity, cell-surface transmembrane receptors fins-like tyrosine kinase (Flt) and fetal liver kinase 1 (Flk-1). Unlike molecules such as basic fibroblast growth factor, VEGF possesses a signal sequence and is secreted from intact cells.[31]

**VEGF in Diabetes Mellitus**

Hypoxia-driven angiogenesis is a crucial pathway in the development of PDR, whereas the leakage of plasma from the small blood vessels in the macula following the disruption of the tight junctions of the blood–retinal barrier is the main factor responsible for CSME. Vascular endothelial growth factor (VEGF) plays an essential role in the development of both PDR and CSME. In ocular tissues, studies have demonstrated that VEGF production is increased by hypoxia in retinal pigment epithelial cells, retinal endothelial cells, retinal pericytes, Muller cells, and both mouse and primate eyes with ischemia-induced retinal and iris neovascularization, respectively. Retinal endothelial cells possess numerous high-affinity VEGF receptors. Recent clinical studies have demonstrated a close correlation between active ocular neovascularization and elevated intraocular VEGF concentrations in patients with diabetes mellitus, central retinal vein occlusion, retinopathy of prematurity, and rubeosis iridis. However, a requirement for VEGF in the retinal neovascular response has not been proven. In this regard, it is significant that anti-VEGF strategies involving intravitreal injections have recently emerged as potential new therapies for PDR and CSME. Lloyd Paul Ayllo et al demonstrate that soluble VEGF-binding chimeric proteins can reduce ischemia-induced retinal neovascularization in vivo without discernible short-term retinal toxicity. Thus, VEGF appears to be important for development of ischemia-induced retinal angiogenesis.[17]

**Results of clinical trials using anti-VEGF drugs**

Several studies have reported very promising results in patients with diabetes. In a prospective, double-blind, multicentre, dose-ranging, controlled trial that included 172 patients with DMO, participants assigned to pegaptanib had better visual acuity outcomes, were more likely to show a reduction in central retinal thickness, and were deemed less likely to need additional therapy with photocoagulation at follow-up (36 weeks) [32]. In addition, most of the participants with retinal neovascularisation at baseline who were assigned to pegaptanib showed a regression of neovascularisation by week 36 [33]. Uncontrolled studies using ranibizumab and bevacizumab have also found a rapid regression of retinal neovascularisation, an improvement in visual acuity and a decrease in retinal thickness, even in non-responders to conventional treatment [34–49]. Moreover, bevacizumab is currently used by many ophthalmologists as a pretreatment of vitrectomy for severe PDR. Nevertheless, larger studies investigating not only the effectiveness, but also the systemic adverse effects of these agents in the diabetic population are still needed.

**Retinal vein occlusions**

**BRVO**

The BRAVO trial, a phase 3 multicenter clinical study, showed that patients receiving 6 monthly injections of 0.3 mg or 0.5 mg ranibizumab experienced a mean improvement of 16.6 and 18.3 letters, respectively, in visual acuity, compared with 7.3 letters improvement in those receiving sham injections. During the second 6 months of the study, all patients could receive 0.5 mg ranibizumab on an as-needed (PRN) basis if they met retreatment criteria; this included the patients who received sham injections in the first part of the study. At 1 year, visual acuity was well maintained in both ranibizumab treatment groups, and there was improvement of visual acuity in the sham group, although there was still statistically significantly less improvement in the sham group than the ranibizumab treatment groups. The percentage of patients who gained three lines (15 letters) of visual acuity was 55.2% and 61.1% in the 0.3 mg and 0.5 mg ranibizumab groups, respectively, compared with 28.8% of patients receiving sham injection.

There were rapid reductions in excess foveal thickness with ranibizumab treatment, and by 6 months there was a substantial mean difference from baseline. The results of the BRAVO study show, therefore, that ranibizumab is an effective treatment for macular edema secondary to RVO in the short term.

In 17 patients with BRVO who completed the study at 24 months, final visual acuity results (17.8 letters improvement from baseline) were similar to those at the primary endpoint of 3 months (16.1 letters improvement).

Foveal thickness measurements with optical coherence
tomography (OCT) in the study reflected the visual acuity results. After 3 monthly injections, all patients with BRVO had a rapid reduction in foveal thickness, and during the PRN phase most remained stable. Recurrence of macular edema was seen only in few patients.

After compiling the results of the 2 year study, it was found that during the second year, patients were eligible for a maximum of six injections of ranibizumab with recurrence of edema.[51] The mean number of injections given was two. Patients seemed to fall into two categories: those whose edema resolved with a few injections and those who continued to need frequent injections.

**CRVO**

The CRUISE study mirrored the design of the BRAVO study. Patients with CRVO receiving six monthly injections of ranibizumab had substantial improvement in visual acuity: 12.7 letters with 0.3 mg and 14.9 letters with 0.5 mg ranibizumab injections, in comparison with 0.8 letters in sham-treated patients. Once those in the sham group were able to receive ranibizumab they also improved, but as in BRAVO they did not achieve the level of improvement seen in patients treated in the first 6 months of the study.

Similar results were seen regarding the percentage of patients who gained at least 3 lines of VA: 46.2% with 0.3 mg and 47.7% with 0.5 mg ranibizumab, compared with 16.9% of sham-treated patients.

In the 2-year follow-up of patients with CRVO, results for the 14 patients who completed the study were quite different from those of patients with BRVO. After the initial three monthly injections, these patients experienced a mean gain of 12 letters at 3 months, but by month 24 the mean gain had decreased to 8.5 letters. This compares with the 17.8 letter gain seen at month 24 in patients with BRVO.[51]

OCT measurements again reflected the visual acuity results, with a mean increase in foveal thickness at month 24 compared with month 3.

As with the BRVO patients, the patients with CRVO were seen every 2 months and received an injection only if their foveal thickness exceeded 250 μm on OCT. These results indicate that this regimen did not maintain visual acuity as effectively in patients with CRVO as it did in those with BRVO. The maximum possible number of injections in year 2 was six, and the mean number given was three.

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Dr Lakshmipriya finished her Ophthalmology from SRMC Chennai and retinal training from AIMS and is working as Junior consultant in Vitreo Retinal Services at Amrita institute of medical Sciences, Kochi
Statistics and Biostatistics - defined

The Webster’s Dictionary defines statistics as ‘the branch of mathematics that deals with the collection and analysis of quantitative data’. By this definition, statistics is an applied scientific discipline whose roots lie in mathematics.

The word statistics can be defined both in the plural and singular sense. In the plural sense, it means ‘numerically stated facts’ or ‘facts expressed in figures’. For example, when the following facts are expressed in figures, they become statistics:

1. The population of India, according to the 2011 Census is 1.25 billion.
2. The total outpatient attendance at the Ophthalmology department at the Amrita Institute of Medical Sciences, Kochi during 2010 was 2.145 lakhs.
3. The number of cataract operations done at Amrita Institute at Kochi in 2010 was 6780.

In the singular sense, it is the ‘science’ dealing with the methods of data collection, their compilation, tabulation and analysis to provide meaningful and valid interpretations. In other words, it deals with the scientific treatment of data derived from individuals.

From these two definitions, we can understand that statistics implies both ‘data’ and ‘methods’. Keeping these two aspects in mind, statistics has been defined in different ways by different authors. Statistics helps in collecting data scientifically, and in organizing, summarizing and presenting the data collected so that valid and meaningful inferences can be drawn with credibility and reliability.

Biostatistics

Statistical methods applied in the fields of medicine, biology and public health are termed as ‘biostatistics’, also called ‘biometry’, which means the ‘measurement of life’. Biostatistics is known by many names—medical statistics, health statistics and vital statistics. Though all these terms may mean the same, one can differentiate between them in the following ways:

Medical statistics: Statistics related to clinical and laboratory parameters, their relationship, prediction after treatment, clinical trials, bioassays, diagnostic analysis, quality control, etc. may be included in ‘medical statistics’.

Health statistics: Statistics related to the health of the people in a community; epidemiology of diseases; association of socioeconomic and demographic variables, personality and behavioural variables, environmental factors and nutrition with the occurrence of various diseases; control and prevention of diseases, promotion of health, etc., are included in ‘health statistics’.

Vital statistics: Statistics related to the vital events in life—birth, illness, death, marriage, divorce, adoption, etc.—their rates of occurrence, causes of increase or decrease in the vital rates, expectation of life at birth and at a given age, etc., are included in ‘vital statistics’.

Population forms the basis of a majority of studies on vital and health statistics. Hence, the study of population, called ‘demography’, also becomes a part and parcel of biostatistics. Accurate information on population with respect to sex, age and other important factors is essential to define the vital statistics rates. Demography pertains to the magnitude, distribution, reasons for increase/decrease in population size, relationship with socioeconomic and environmental factors, etc.

Uses of Medical Statistics

Statistical methods are very widely used in both research and administration in almost all fields, such as medicine, biology, public health, agriculture, industry, economics and meteorology. One common question medical students or practitioners generally ask is: why should they learn statistics? Generally, a biology or medical student would like to keep mathematics and formulae at a distance. Hence, after choosing medicine as their career, they wonder why they are asked to take the trouble of learning mathematics/statistics. The following sections may provide an answer to this question.

Uses of statistical methods in general

1. Statistical methods, in general, are needed for the following tasks:
2. For gathering (collecting) medical and health data
may be positively associated with smoking habits and diabetes may be positively associated with the type of job (sedentary type or field work), while tuberculosis may be negatively associated with socioeconomic class. The chance of developing lung cancer is higher in heavy smokers than in non-smokers or mild smokers and the chance of developing diabetes is higher in those with sedentary type of work compared to those engaged in work requiring movement and labour.

(4) To develop new, more effective drugs and treatment methods for various diseases and to predict various outcomes (improvement, cure, living or dead) after treatment, based on various factors. This branch of study is called ‘clinical trials’. Treatment ‘A’ may be better than treatment ‘B’ or no treatment in improving the condition of a disease of the patients, controlling for the effect of various factors, such as age, behavioural habits, diet, etc. To test the efficacy of new vaccines for the prevention of diseases. This branch of study is called ‘prophylactic trials.’

(5) To collect data scientifically on vital events in life (birth, death, fertility, morbidity), to estimate vital statistics rates (birth, death, fertility, morbidity rates) and to evaluate the expectation of life (number of years expected to live) at birth and at various ages by constructing the Life Table based on mortality data. This branch of statistics is called ‘vital statistics’.

(6) To estimate the potency and relative potency of drugs, to determine ED50 (the effective dose of the drug at which 50% of those responded positively (i.e., showed improvement) and determine the route and frequency of administration of the drug to give the maximum benefit to the patients. This branch of study is called ‘biological assays’. If the relative potency of drug A compared to that of drug B is 1.2, then 1 unit of drug A is equivalent to 1.2 units of drug B. Relative potency is computed by applying specific statistical methods;

(7) To estimate the probability of survival after treatment for a specified period in chronic diseases such as cancer and AIDS. This branch of study is called ‘survival analysis’. Based on the treatment and mortality data of cancer patients, the chance of survival of a patient after a certain period of treatment can be computed by applying survival analysis methods and it can be compared with the corresponding chance of survival after treatment with another drug.

(8) To assure and maintain the quality of drugs and laboratory, surgical and medical instruments, and equipments. This branch is called ‘quality control analysis’.

(9) For the validation of new, efficient and economic screening and diagnostic tests in comparison to those already existing. For example, validation of the sputum test with respect to X-ray for detecting tuberculosis and validation of the
HbA1c test with respect to fasting and postprandial blood sugar levels for detecting diabetes. This is called ‘validation analysis’. Validity parameters such as sensitivity, specificity, predictive values of positives & negatives and accuracy are computed for this purpose.

(10) To study the genetic composition of a population and the changes in the composition with respect to factors such as mutation, migration, etc., and their impact on the health status of the people. This branch is called ‘statistical genetics’. 

(11) To ensure that the maximum benefit of diagnosis, treatment methods and prognosis reaches the population with minimum cost, based on available resources. This branch is called ‘health economics and operational research. 

In some of the later chapters, the statistical methods applied to study these aspects will be discussed with examples.

Branches of Statistical Methods

Statistical methods can be broadly divided into two branches—design methods and analysis methods.

Design methods

Design methods deal with the methods of collecting data scientifically. The role of statistics is not restricted to analyzing data, but also involves planning the study scientifically and executing it properly. Many researchers are tempted to give more emphasis to the statistical analysis of data applying sophisticated methods, while giving lesser or no importance to the design of studies. If the design of the study is defective, the results of the study will have limited validity or not be valid at all. Hence, designing the study needs care as much for analyzing the data.

In most situations, the data are not collected as complete coverage, but as sample coverage. In case it is possible, complete coverage is ideal. However, in most cases, it is not practically possible or feasible because of a lack of time, budgetary restrictions and the large number of personnel required for such studies. For example, for estimating the percentage of population of Kochi who have partial blindness, within a short period and with a comparatively small budget, it may not be practicable to screen the entire population of Kochi for partial blindness. In such situations, data are usually collected from a small representative portion of the total. This helps in getting results in much less time, with fewer resources and personnel. The study element in ‘totality’ (complete coverage) is called ‘population’ or ‘statistical universe’ and the small representative portion (sample coverage) is called ‘sample’. That is, the population comprises the study elements in totality while the sample is a small portion of the population, representative of various characteristics, which may influence the study variables. One of the important aspects of the design of a study is determining the minimum sample size required to be selected from the population and the appropriate method of selection so as to ensure the ‘representativeness’ of the sample. Apart from this, choosing the appropriate study method (design)—whether it is an observational or an experimental study and whether it is a one-time (cross-sectional) or follow-up study (longitudinal)—also form part of the design of a study.

Analysis methods

Statistical analysis methods are of two kinds—descriptive methods and inference methods.

Descriptive methods: Descriptive methods include the statistical methods used to summarize the data collected in terms of statistical tables, diagrams and graphs and certain summarizing parameters such as averages, variation and correlation. The data collected can be more clearly understood and interpreted in terms of these summarization methods. For example, a table giving data on completely blind persons with respect to their sex and age group may show that 60% of the blind were males; among the males, more than 70% were 50 years or order while among the females, 80% were in this age group.

Inference methods: Inference methods include the statistical methods used to generalize the results obtained from the sample selected for the entire population. ‘Science’ is an investigation towards truth, supported by experimental evidence. However, experiment and induction do not always lead to the truth. For example, two scientists may arrive at two different conclusions from the same experiment. Statistics provides the means of measuring the amount of subjectivity that goes into the conclusions, in terms of probability. Based on a theoretical model, the probabilities of the various possibilities of the experiment can be estimated and it can be determined whether the effect of the treatment is real or could have occurred by chance alone.

However, statistics cannot prove anything with 100% confidence. It is only a powerful and reliable tool to get as close to the truth as possible. Hence, even if it is concluded that the better outcome of the treatment is real, there will be a chance element (error) in that statement. In statistical inference, the chance element can be estimated and can be made as small as possible by choosing the design of the study appropriately. This is called the ‘p’ value, or level of significance, or type I error or alpha. While generalizing the results obtained from the sample for the population, this chance element has to be stated.
Studies based on a sample provide results for the sample only; but, what is required is the results for the population. Inference methods help us to generalize the results obtained from the sample for the entire population with a certain amount of confidence. For example, if the estimate of the percentage of persons affected by cataract in a Town was 10% based on a sample study, this estimate can be refined by attaching a specific confidence, say 95%, that the true (population) value lies between, say 8%, and 12%. This means that there is a chance of 5% that the true value may lie beyond these two limits. If 40% of 50 conjunctivitus patients receiving the standard eye drops are cured, while 60% of a comparable group of 50 conjunctivitus patients receiving a new eye drop are cured, and if the statistical test of significance shows that this difference of 20% in the cure rate is statistically significant at a chosen level of significance, say 5%, (95% confidence), then there is a 5% chance that this difference is not due to the better efficacy of the new drug, but, just by chance. The concepts of statistical significance, chance and confidence will be discussed in detail in later chapters.

Some Basic Statistical Concepts
It is important and essential to get familiar with a few basic statistical concepts as a starting point.

Scales of Measurements
There are two types of data characteristics one can study—constant and variable

Constant
A constant is a value that does not change with any situation. For example, the value of \( \pi \) is 22/7, which does not change with time, place or person. Similarly, the value of \( e \), the base of the natural logarithm is 2.7183. This is a constant and the value will not change in any situation. These are called mathematical constants.

Variable
In contrast, the value of a variable changes. A variable is a characteristic that can take on different values with respect to a person, time or place, or any other factor. For example, blood pressure, height, weight, blood group, cholesterol level, pulse rate, circumference of eye balls, thickness of cornea, severity of illness and blindness (partial or complete), the grade of a student in an examination are variables. Basically, there are two types of variables: discrete and continuous.

Discrete (categorical) variable: If the characteristic is classified according to a group, class or category, it is called a ‘discrete’ variable. Examples of discrete variables are blood group (A, B, AB and O), status of a disease (severe, moderate and mild), socioeconomic class (rich, middle class and poor), grades in examination (excellent, good, average and poor), sex (male and female), blindness (partial, complete) and diet (vegetarian and non-vegetarian). In this type of variable, the number of persons falling in each group of the variable is counted. Hence, this type of variable is also called ‘countable variable’.

There are two types of discrete variables: ordinal and nominal. If there is an order in the classification of the groups of the variable, it is called an ‘ordinal’ and if no order is possible in the classification, it is called ‘nominal’. Status of disease, grade in examination and socioeconomic class are ordinal variables while sex, diet and blood group are nominal variables.

Another type of discrete variable is the one classified into groups, but having some numerical value. Families classified according to size (one member, two members, three members, etc.) or number of children (no child, one child, two children, three children, etc.) are examples of discrete variables, which can be classified into groups assigned a numerical value. If there are only two groups, such as sex, the variable is called ‘binary’ or ‘dichotomous’. If there are more than two groups, it is called ‘polychotomous variable’.

Continuous (measurable) variable: If the characteristic is measurable in units of measurement carrying a numerical value, it is called a ‘continuous’ variable. Examples of continuous variable are weight (kg), height (cm), age (years or months), blood pressure (mmHg), time (hours, minutes and seconds) and circumference of eye ball. This type of variable can have decimal point values. The weight of an individual can be 50.8 kg and the height 170.3 cm. Since any interval of this type of variable can still be refined, it is called a continuous variable. Even between 50.5 and 50.6, a value such as 50.57 can be obtained.

Observation and Data
Each value of a variable recorded is an ‘observation’ and a set of observations of one or more variables form the ‘data’. If the values of the variable in the population are not affected by any external factor, the data are homogeneous; but if they are affected by some external factor, the data are heterogeneous. Data on birth-weights of babies born to women from a poor socioeconomic class alone are homogeneous. But data on birth-weights of babies born to women from high, middle and poor socioeconomic classes are heterogeneous.
Data pertaining to discrete variables are called ‘qualitative data’ and those pertaining to continuous variables are called ‘quantitative data’. Most of the data related to biology and medicine could be quantitative data and those for public health and sociology could be qualitative data.

**Parameter and Statistic**

‘Parameter’ is the statistical characteristic related to the population and ‘statistic’ is the statistical characteristic related to the sample. If the percentage of population affected by cataract is 10%, it is a parametric value. If the corresponding value in the sample selected randomly from the population is 12%, it is a statistic value. The former is usually represented as ‘P’ and the latter as ‘p’. Similarly, if the mean systolic blood pressure (SBP) of the male population is 120 mm Hg, it is the parametric value (μ) and if the SBP of a sample of males selected randomly from the population is 122 mm Hg, it is the statistic value ( ). What we are able to get from studies based on samples are the statistic values of the statistical characteristics and those values will be estimates of the corresponding parameters. When the sample size increases, the statistic value estimated will be as close as possible to the population parameter values and it is then said to be an accurate estimate of the parameter.

**Ratio, Proportion and Rate**

The parameters ratio, proportion and rate are computed for summarizing data related to discrete variables (qualitative data). Many people consider all these terms to mean the same, but in reality they are different.

**Ratio**

Ratio is obtained simply by dividing one quantity by another, without implying any relationship between the numerator and denominator, i.e., the numerator is not a part of the denominator. Examples are patient/doctor ratio, patient/nurse ratio and student/teacher ratio. If in a hospital there are 100 doctors and 300 nurses, then the doctor/nurse ratio is 100/300 or 1:3. If there are 1000 students in a school and the number of teachers is 50, then the teacher/student ratio is 50/1000 or 1:20.

**Proportion**

Proportion is a type of ratio in which the numerator is included in the denominator. For example, if there are 1000 males and 900 females comprising a total population of 1900, the proportion of females in the population is: Proportion (P) = 900/(1000+900) = 900/1900=9/19

This is usually expressed as a percentage or in multiples of 10s such as 1000, 10 000, 100 000, etc., depending on the number in the numerator relative to the denominator. In this example, the percentage of females in the population is (9x100)/19=47.4%. Similarly, the percentage of males in the population is 52.6%. If the number in the numerator is small (number of glaucoma cases) compared to the denominator (total population), it is expressed per 1000 or 10,000 or even 100,000 to avoid expressing it in terms of decimal point. For example, it would be better to express a value as 50 per 10000 instead of 0.5 %.

**Rate**

Rate is a ratio in which a distinct relationship exists between the numerator and denominator and, most essentially, a measure of time is an intrinsic part of the denominator. Rate requires the numerator, e.g., cases of a disease, acquired over a specific time interval and a denominator, e.g., the population which did not have the disease, observed during the same time interval. For example, crude death rate is defined as the number of deaths that occurred during a specific period of time, say one year, divided by the total mid-year (as on 1 July) population of the same area and over the same period of time. It is expressed per thousand of the population. Other common examples of rate are: typing speed (number of words/minute) and speed of a car (kilometers/hour).

**Variation**

One of the most important and basic concepts in statistics is ‘variation’. It can even be said that statistics is nothing but the study of variation. If there is no variation, there is no statistics. Variation is inherent in nature. No two things are alike. There may be at least a slight variation even under the most homogeneous conditions. In studying the blood pressure of persons there could be variation between individuals of the same sex, age and many other factors (individual variation). Variation could also be there in the same person when the reading is taken at two different times/places (within variation).

**There are basically four types of variation:**

1. Biological variation
2. Experimental variation
3. Real (environmental) variation
4. Sampling variation.
Biological variation

Variation that is normal or natural or due to a chance or random occurrence within biological limits is called biological variation. This type of variation can be determined statistically. Variation in blood pressure, height, weight, haemoglobin level, etc., of individuals of the same age, sex, socioeconomic status, environment, etc., could be of biological variation type.

Experimental variation

Variation due to the observer, instrument or method is called 'experimental variation'.

Observer variability: Variation due to the persons measuring the parameter or recording/collecting the information is called 'observer variability'. Blood pressure or pulse rate or cholesterol values of the same person measured by two observers may not be the same because of differences in their experience, training or concentration. Information obtained by two investigators from the respondent on an item can also vary because of the way the question is asked (age in completed years or actual age; household income per year including only the income of the main earning member in the house or including the income of each earning member in the family and including or excluding income from other sources, etc.). This type of variation can be minimized by giving systematic and uniform training to observers in measuring or reading the variable values, or asking relevant questions.

Instrument variability: Variation occurring due to the type of instrument or equipment used to measure the parameter is called 'instrument variability'. Blood pressure of a person recorded with a sphygmomanometer and aneroid instrument, or two sphygmomanometers may vary, as may the weight of a child using two types of weighing machines (beam balance and spring balance or two beam balance machines) because of the differences (may be minute) in the instruments. This type of variation can be minimized by the standardization of the instruments to be used for measuring the parameter and using accurately tested instruments giving reliable (consistent) readings.

Method variability: Variation may occur with the laboratory procedures used for the estimation/measurement or recording of parameters such as haemoglobin and cholesterol level, and the nutritional status of children due to the different procedures, chemicals, reagents or measurements used. This type of variation can be minimized by the standardization of the techniques, measurements, chemicals or reagents to be used for measurement or recording.

Environmental/behavioural factor/intervention variation

This is the real variation which is beyond the limits of normal variation. This type of variation is not by chance or random. Biological variation is mainly anatomical or physiological in nature. However, pathological variation is real variation and is mainly due to environmental factors. Variation in the blood pressure or cholesterol level of individuals according to their smoking habits, type of job (sedentary or active) and diet, variation in the nutritional status of children in accordance with their socioeconomic status, parents’ education or diet will not be biological. This type of variation occurs due to the influence of the afore-mentioned environmental and behavioural factors and can be minimized by suitable intervention such as giving treatment by administering a drug or by surgery; quitting smoking; exercising; eating nutritious food; improving the economic standard, etc., as the case may be.

Sampling variability

Variation occurring due to the method of sampling and/or sample size is called ‘sampling variability’. This type of variation cannot be avoided in studies based on samples. This results in error and can be measured statistically. Variation of this type can be minimized by adequately increasing the sample size and/or by adopting an appropriate sampling method.

Statistics is the study of variability and chance. There will be variation in the results obtained from different samples. For example, the prevalence of malnutrition in children from one study of a sample of children may be 10%, while from another study of a sample of children from the same population, it may be 12%, and so on. Similarly, in a clinical trial, the difference in the improvement rate with the standard drug and the new drug may be 5% from one sample study and 6% from another study, and so on. This variation is called random or chance variation. Chance/uncertainty is measured by probability. No conclusion can be drawn with 100% certainty (confidence). A statistical inference method enables us to quantify the extent to which the chance variability can affect the results by computing the probability of the result occurring by chance alone. In clinical trials, if the improvement with a new drug is 80% and that with the standard drug is 60%, to determine whether this difference is real (the additional
improvement rate of 20% is due to the better effect of the new drug compared to that of the standard drug) or not, an appropriate statistical test is performed and the probability that the additional improvement rate is due to the effect of the new drug compared to that of the standard drug is computed. This probability value is simply called as the 'p' value and it varies from 0 to 1. As a rule of thumb, if the p value is less than 5%, then it is concluded that the difference is not by chance, but real and that the difference is statistically significant with 95% confidence. The confidence can be increased (the proportion of chance can be reduced) by increasing the sample size of the study.

Accuracy, Precision and Unbiasedness of the Estimate

Ideally, any measurement made is expected to be accurate. An accurate measurement is defined as one that is precise and unbiased. These terms are not the same and each term has a different meaning. Accuracy is the closeness of a measured or computed value to its true value and precision is the closeness of repeated measurements of the same variable to each other. Precision may also be called as reliability, repeatability, consistency, stability and reproducibility.

If the estimated values of the parameter from different samples are concentrated around a point, then it is precise. If that point is the true value of the variable, on an average, it is also unbiased and may be accurate, considering the central point to be an estimate of the true value. That is, on an average, it measures consistently and near to the true value and is, hence, accurate. If the grouping is not around the central value, it causes a systematic error and becomes biased. The reason for this difference may be due to some specific factor. If the measurement is made by two persons, one having a lot of experience and the other not much experience, there may be consistency in the values measured by each person separately, but the measurements made by the experienced person may be unbiased and, hence, accurate. The measurement made by the fresher may also be consistent, but may be biased since his values may be concentrated around a point, which may not be the central point (true value). The difference between the true value and the value obtained by the persons, on an average, is called 'bias'. In the case of an experienced person, the bias may be negligible while in the case of an inexperienced person, the bias may be high.

In the next issue descriptive statistical methods will be discussed with examples of tabulation, drawing of diagrams & graphs, computation of statistical parameters such as averages (arithmetic mean, median, mode and geometric mean), range, standard deviation and coefficient of variation and correlation between variables and their prediction equations.

Books for further reading:

Cataract Surgery in Eyes with Small Pupil

One of the most challenging situations in ocular surgery is cataract surgery in eyes with nondilating pupil. There is an increased risk of complications like iris trauma, anterior capsular tear, posterior capsular rupture, zonular dialysis and vitreous loss during cataract surgery in eyes with small pupil. A pupil which doesn’t dilate beyond 4mm may be called a small pupil. These cases have to be evaluated properly before taking them up for surgery. Small pupil can also be associated with floppy iris syndrome. Always ask for use of alpha-1 blocker medications as it can be associated with Intraoperative Floppy Iris Syndrome (IFIS). The intraoperative diagnostic triad of this syndrome is fluttering and billowing of the iris tissue, a tendency for iris prolapse, and progressive constriction of the pupil during surgery. Also look for the presence of any ocular pathology that can interfere with good dilation such as pseudoxefoliation syndrome, posterior synechiae or pupillary membrane and plan accordingly.

If the pupil doesn’t dilate by any of the pharmacological methods, one can plan to undertake the surgery leaving the pupil as it is or use one the many mechanical techniques for dilating the pupil. We are first showing a video of phacoemulsification in a case with small pupil without using any mechanical dilatation of the pupil. This is followed by a video on use of iris retractors in phacoemulsification. The next three videos are on manual SICS, the first one without mechanical dilation and the next two using iris hooks. The last video demonstrates the use of Malyugin ring.

In the first video in which the surgeon does phacoemulsification without using any of the pupil dilating techniques, capsulorhexis is done taking care to prevent capsulorhexis extension to the periphery. He makes sure that are no dog ears when completing capsulorhexis to prevent anterior capsular tears which can extend to the posterior capsule during nucleus rotation and fragmentation. All nucleus manipulations are done in the pupillary area. During phacoemulsification he uses a low bottle height with very low suction and flow rates. This prevents pupil getting caught in the phaco tip. He also doesn’t take the phaco tip to the papillary margin. Doing endocapsular lens nucleus fragmentation is much safer in eyes with small pupil as the areas of the highest fluidics currents are then located inside the capsular bag, away from the corneal endothelium and iris. During cortex wash the pupillary margin is manipulated with the dialler for better view. A Y pusher may also be used for this. http://www.youtube.com/watch?v=XZ0XwTlMQ

Pupil can be mechanically dilated either by mechanical stretching of the pupil or by using iris hooks or pupil expansion devices. If posterior synechiae are present they should be released and if pupillary membrane is present it should be removed. Mechanical stretching of the pupil can be done with Sinskey hooks, spatulas or Kuglen hooks or any other device which can be used for stretching and pulling/pushing the pupillary margin. The hooks are either placed through two paracentesis or one through the paracentesis and the other through the main wound. The hooks are used to pull or push the pupillary margin in opposite directions to stretch the pupil. The stretch is held for a few seconds. This causes microscopic sphincter tears which enlarge the pupil. A dispersive viscoelastic may then be used to push the pupil open and maintain the dilatation. Partial-thickness cuts can also be made to iris sphincter with microscissors to cause papillary dilatation. But these techniques for papillary dilation can result in bleeding and pigment dispersion, instability of pupillary margin and enlarged atomic pupil postoperatively.

The commonly used mechanical iris retainer devices for pupil stretch/expansion are Iris hooks and Malyugin ring. Iris hooks can help in both SICS and phacoemulsification while Malyugin ring is not ideal for SICS. In cases with shallow anterior chamber it is better to use Iris hooks. Iris hooks are also to be preferred if you have to use Conn rings. It was Richard J. Mackool, who first described a four-point iris retractor configuration for phacoemulsification. He used metal iris retractors which were connected to small blocks of titanium to allow stabilization of the hooks during iris retraction. Eugene de Juan, Jr., MD, and Dyson Hickingbotham, MD, enhanced this method by introducing flexible iris retractors. Three, four or even five iris retractors are used through limbal paracentesis.

The second video is of phacoemulsification done on a case of Primary Angle Closure Glaucoma with cataract which had already undergone trabeculectomy. Four paracentesis were made without damaging the bleb for inserting the iris hooks. The paracentesis for inserting the iris hooks are to be made as posterior as possible at the limbus. The paracentesis are made short and angled slightly down in such a way that when the hook is placed in the eye, it is aimed towards the iris. Hooks are then introduced into the anterior chamber
and engaged to the pupil margin and then retracted to dilate pupil. When it was seen that the lens was subluxated the surgeon uses iris hooks to stabilise capsular bag.

The most difficult part in manual SICS in a case with small pupil is the capsulorhexis. It has to be fashioned under the edge of the iris without direct visualization and the capsulorhexis has to be larger than for phacoemulsification. In the third video clip you can see this. At one point capsulorhexis gets lost under the iris. It is then retrieved using a capsulorhexis forceps. Since the surgeon is not sure as to how big the capsulorhexis is the hydrodissection is done very gently. The nucleus is rotated into the anterior chamber using a sinskey hook and then delivered out using irrigating vectis. Since the nucleus has to squeeze out through a small pupil the pupil becomes even more constricted after nucleus delivery as seen here. This makes cortex wash extremely difficult. http://www.youtube.com/watch?v=OUwtoHB8KLM

These difficulties can be overcome if iris hooks are used as seen in the next video clip. Four stab incisions are made for inserting iris hooks. Iris hooks are then inserted and pupil is stretched. The capsulorhexis can then be done under full visualisation. If the capsulorhexis needs to be enlarged, it also can be done under full visualisation. The two hooks towards the tunnel are loosened during nucleus delivery so that iris doesn’t get caught in the vectis during nucleus delivery. http://youtu.be/YX9WPSmOsDQ

If there is a pupillary membrane is removed by teasing it from the pupillary margin. This manoeuvre itself makes the pupil larger. In the fourth video this is shown. This is followed by insertion of iris hooks. http://www.youtube.com/watch?v=Em0uVNqztww

Use of iris hook can lead to irregularly shaped atonic pupil with poor cosmetic result and photophobia. This can be overcome by use of pupil expansion rings. Currently there are a number of pupil expansion devices in the market: the Malyugin Ring from MicroSurgical Technology, the Perfect Pupil device from Milvella, the Oasis Iris Expander from Oasis Medical, Inc.(7.0mm diameter polypropylene ring), the Graether pupil expander from Eagle Vision (Silicone pupillary expansion ring - less sphincter trauma), the Siepser Iris Protector ring (hydrogel ring which expands on hydration and has flanges on its inner surface) and the Malyugin Ring System consists of a presterilized single-use holder containing the ring and an inserter. The device comes in two sizes: 6.25 and 7 mm. The same insertion device is used for implantation and removal of both versions. The advantage of the smaller ring is that it is easier to insert and retract while the 7-mm ring can be used if the pupil starts off bigger or in IFIS cases. Cortical aspiration is also easier with the 7-mm Malyugin ring.

The sixth video clip shows insertion of Malyugin ring for phacoemulsification. Injector tip contains a blunt hook. The surgeon grasps the proximal scroll with the blunt hook. The hook is then retracted by pulling the sliding tab which pulls the ring into the shaft of the injector. When the ring is injected, the leading scroll is directed to capture the opposite pupil margin. The rest of the ring is then deposited into the anterior chamber. The remaining three scrolls are then engaged to the pupil margin with sinskey hook or Malyugin ring manipulators. After implantation of the IOL, the ring scrolls are disengaged by a sinskey hook. Proximal scroll is grasped with the hook of the injector and refolded inside its shaft and removed.

Though cataract surgery in eyes with small pupil is extremely challenging, modifications in surgical technique can help the surgeon avoid the possible complications. An experienced surgeon can do cataract surgery in an eye with small pupil without any further expansion of the pupil. But use of pupil stretching/expansion devices will definitely make the surgery easier and safer.

REFERENCES


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Fundus Fluorescein Angiography

Principle
Fluorescence is the property of certain molecules to emit light of a longer wavelength when stimulated by light of a shorter wavelength. The blue part of the spectrum (490 nm) is the excitation peak for fluorescein, where the maximal absorption of light occurs. Molecules stimulated by this wavelength will be exited to a higher energy level and will emit light of about 530 nm, which is in the yellow-green spectrum. Fluorescein angiography involves photographic surveillance of the passage of fluorescein through the retinal and choroidal vasculature following intravenous injection.

Equipment
An ideal fundus camera should be able to reproduce well-resolved photographs when the media are clear, as well as hazy, and even when the pupil is small, there is poor circulation or in cases of irregular or extreme refractive errors. There are 2 types of filters in cameras: Cobalt blue excitation filter-emits blue light which excites the fluorescein molecules in the choroidal and retinal vasculature, emitting light in the yellow-green wavelength. Yellow-green barrier filter-blocks blue light from the eye, allowing only emitted yellow-green fluorescent light to pass through. The image is captured via the charge-coupled device (CCD) of a digital camera, which permits immediate picture availability, easy storage and access, image manipulation and enhancement.

Sodium fluorescein is an orange water-soluble dye, containing 500 mg of fluorescein as 5ml of 10% fluorescein or 3ml of 25%, that, when injected intravenously, remains largely intravascular and circulates in the blood stream. 70-80% of fluorescein molecules get bound to serum proteins, the rest remaining unbound.

Technique
Rule out any contraindications to using fluorescein such as severe allergy, renal diseases, pregnancy, moderate-severe asthma and significant cardiac disease. Cannulate the patient in the antecubital vein. Seat the patient in front of the camera and obtain colour photographs. ‘Red-free’ image is captured. Fluorescein is injected over the course of a few seconds and images are taken 5-10 secs later at approximately 1 second intervals through desired phases. Late photographs may be taken 10 mins later to show leakage and occasionally after 20 mins. Stereo images may be helpful to demonstrate elevation, and are usually taken by manual repositioning of the camera sideways or by using a special device (a stereo separator) to adjust the image.

Certain adverse effects like discoloration of skin and urine, and nausea, are very common during an FFA and mostly invariable. Dangerous side effects like vasovagal attacks, anaphylaxis, thrombophlebitis have to be looked out for. Very rarely, death may also occur. It is essential to have resuscitation equipment and trained personnel ready while doing a fluorescein angiogram to deal with any severe anaphylaxis.

Phases of the angiogram
- **Choroidal (pre-arterial) phase:** 9-15 secs after dye injection-patchy lobular filling of the choroid due to leakage of free fluorescein from the fenestrated choriocapillaris.
- **Arterial phase:** one second after the onset of choroidal fluorescence and shows retinal arteriolar filling and the continuation of choroidal filling.
- **Arteriovenous (capillary) phase:** complete filling of the arteries and capillaries with early laminar flow in the veins in which the dye appears to line the venous wall leaving an axial hypofluorescent strip.
- **Venous phase:** Laminar venous flow progresses to complete filling, with late venous phase featuring reducing arterial fluorescence.
- **The late (recirculation) phase:** The intensity of fluorescence becomes weaker although the disc shows staining, with each succeeding wave of the dye.
- **Fluorescein is absent from the retinal vasculature after about 10 mins.**

Interpretation of the angiogram always to be done in conjunction with colour photo and each abnormality to be followed up across the phases of the angiogram to see its evolution.

**Hypofluorescence**
It is the reduction or absence of normal fluorescence, which appears as an abnormally dark area on the positive print of an angiogram. It may be due to (a) optical obstruction (‘masking’ or blockage) of normal density of fluorescein in a tissue or (b) inadequate perfusion of tissue.

a) In blocked fluorescence, fluorescein is present, but cannot be seen. It can be a 1) Blocked retinal fluorescence wherein any vitreous hemorrhage, opacity or preretinal hemorrhage, obscures details of the retinal vasculature.

b) In vascular filling defect, the fluorescein is not present in an area of the fundus vasculature. It may be due to a vascular occlusion, which may involve the retinal arteries, veins or capillaries (‘capillary drop-out’) or the choroidal circulation. It may also be due to loss of vascular bed as in myopic degeneration and choroidemia.
Filling defects may due to conditions that can be total or partial. When the obstruction is complete (occlusion) or the vascular tissue is atrophied completely, the hypofluorescence is complete and lasts throughout the angiogram. When the obstruction is only partial, the vascular fluorescein filling is delayed or reduced relative to corresponding areas that fill normally. Since retinal vessels are seen more readily on an angiogram, it is easier to recognize a filling defect due to a retinal vasculature pathology than a choroidal one.

The most common diseases that are associated with retinal vascular filling defects are retinal vascular occlusions and diabetic retinopathy.

The best way to distinguish between a blocked fluorescence and filling defect is ophthalmoscopic examination or correlation with a corresponding colour photo. When blood, pigment or exudates can be seen ophthalmoscopically corresponding to the area of hypofluorescence, the material is masking the fluorescence. When the fundus is devoid of any material ophthalmoscopically, one must assume that the fluorescein has not perfused the vessels and the abnormal hypofluorescence is caused by a vascular filling defect.

Hyperfluorescence
It is any abnormally light area on the positive print of an angiogram i.e, an area showing fluorescence in excess of what would be expected on a normal angiogram.

Fluorescein almost completely empties from the retinal and choroidal vessels about 15 mins after injection. There are four types of late extravascular hyperfluorescence leakage that occur in the normal eye. They are:
- Fluorescence of the disc margins from the surrounding choriocapillaris
- Fluorescence of the lamina cribrosa
- Hypofluorescence of the choroid due to preretinal hemorrhage in PDR

Causes of abnormal hyperfluorescence-
1) Pre-injection fluorescence(autofluorescence and Pseudofluorescence)
2) Transmitted fluorescence(window defect)
3) Leakage
4) Pooling
5) Staining

Preinjection hyperfluorescence is that which can be seen before fluorescein is injected and is caused by structure that naturally fluoresce (autofluorescence), for example optic nerve head drusen, .Poorly matched filters cause pseudofluorescence, which occurs when non-fluorescent reflected light visible prior to fluorescein injection passes through the filters due to the overlap of wavelengths passing through the excitation then the barrier filters. This occurs more commonly when the filters are wearing out.

Transmitted fluorescence occurs in the early or vascular stage of the angiogram. It is an accentuation of normal choroidal fluorescence. This occurs when there is a pigment epithelial window defect caused by atrophy or absence of the RPE as in atrophic age-related macular degeneration, RPE tears. This results in unmasking of the normal background choroidal fluorescence, characterized by very early hyperfluorescence, increasing in intensity and fading without changing size or shape.

Leakage is said to occur when there is hyperfluorescence that increases in size and intensity across the phases of the angiogram. Leakage can occur due to breakdown of either
the inner bloodretinal barrier which is endothelial tight junctions (neovascularisation, vasculitis) or the outer blood retinal barrier constituted by the retinal pigment epithelium and bruchs membrane (central serous retinopathy and choroidal neovascular membrane). When fluorescein enters the vitreous, it is known as vitreous leak, which creates a diffuse white haze in the late phase of the angiogram. It may occur due to neovascularisation into the vitreous cavity, intraocular inflammation or intraocular tumors. Disk leak has said to occur when the optic nerve head hyperfluoresces.

Hyperfluorescence-Leakage in CSR

Pooling of fluorescein in an anatomical space occurs due to breakdown of the outer blood-retinal barrier (RPE junctions), characterised by early hyperfluorescence that increases in intensity, but not in size.

- in the subretinal space as in central serous retinopathy
- in the sub-RPE space in PED

Staining is a late hyperfluorescence due to prolonged retention of the dye in tissue such as that occurs in drusen, fibrous tissue, exposed sclera and the normal optic disc. Arm to retina time-the time taken from injection in the cubital vein for fluorescein to enter the retinal circulation. Usually 9-15secs and it is delayed in carotid occlusive disease and in poor general circulation due to any cause.

In conclusion fluorescein angiogram is an extremely useful tool in retinal disease diagnosis and management that greatly enhances our understanding of many retinal and choroidal pathologies.

Autofluorescence-optic nerve head drusen

Hyperfluorescence-staining-optic disc in late phase

Hyperfluorescence-pooling in PED

Hyperfluorescence-window defects

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Purpose
To assess long term visual outcomes for type 2 diabetic patients receiving early vitrectomy and endolaser for first episode of severe vitreous hemorrhage (VH).

Methods
Review for 150 eyes that had early pars plana vitrectomy and endolaser within 6 months of first episode of VH with at least 5 years follow up, for postoperative and long term visual acuities (VA) and final retinopathy classification.

Results
Mean postoperative VA was 0.98 log MAR, improving to 0.59 log MAR (p= .0002) at 2 weeks postoperatively and 0.56 log MAR (p= .0008) at most recent clinic appointment, where only 10 patients(10%) had active proliferative retinopathy. Recurrence of VH needing additional laser fill-in was seen in(10%)

Conclusion
Type 2 DM with VH can obtain long term improvement in VA and stabilization of their retinopathy after early vitrectomy and endolaser.

Diabetic retinopathy is a leading cause of vision impairment ¹. The treatment strategies for diabetic retinopathy developed over the last several decades are, in part, based on diabetes control, laser photocoagulation and vitrectomy ²,³. The efficiency of laser photocoagulation for proliferative diabetic retinopathy (PDR) was demonstrated in the Diabetic Retinopathy Study (DRS) ³. Treatment with photocoagulation, reduced the rate of severe vision loss (visual acuity of worse than 5/20) of at least 4 months duration, by 50 %. This treatment became the standard of care for patients with PDR. The results from Early Treatment Diabetic Retinopathy Study (ETDRS) ⁴ suggested that scatter laser photocoagulation should be considered for all eyes with severe non proliferative diabetic retinopathy or worse and not for those with mild to moderate non proliferative diabetic retinopathy. For patients with type 2 diabetes, early treatment at the severe non proliferative stage rather than waiting for the stage of high risk proliferative retinopathy is considered because the rate of severe visual loss was reduced by more than 50 % in eyes treated with early laser photocoagulation. ETDRS also suggested that focal laser photocoagulation in eyes with clinically significant macular edema reduced the rate of moderate vision loss by 50 % ⁴.

The results of the Diabetic Retinopathy Vitrectomy Study (DRVS)⁵, a randomized clinical trial of vitrectomy for eyes with dense vitreous hemorrhage and for eyes with very severe PDR, showed a greater chance of recovering good vision with vitrectomy. With the improvement in vitreectomy techniques and instrumentation, the visual results have markedly improved since these early trial. DRVS has clearly demonstrated the advantage of early surgical intervention in the more severe cases in the type 1 DM population. Endolaser has been shown to further improve long term visual outcome. There was no evidence to suggest that this benefit is obtained in type 2 DM population. The improved visual outcome in type 1 DM was put down to better macula function, less development of lens opacity and lesser susceptibility to untoward events, it was also suggested that the younger type 2 DM patients had greater severity of new vessels, fibrous proliferations and vitreoretinal adhesions⁶.

It is broadly accepted now that early vitrectomy in a non clearing VH is useful for not only clearing the visual media but also allows retinal observation laser photoacoagulation and may stabilize the retinopathy. Our study shows that type 2 DM patients can obtain sustained improvement in VA and stabilization of there proliferative retinopathy after early vitrectomy and endolaser that is comparable to the type 1 population, a finding that was, not investigated in the DRVS. The reason for this difference is said to be multi factorial - earlier interventions, better instrumentation, adjuvant operative endolaser and pan retinal photocoagulation before VH are all likely to have a role in better long term outcomes with regard to VA as well as retinopathy classification, compared with the DRVS population.

Each patient's particular situation and disease state must be carefully considered; however there is growing evidence that early vitrectomy for VH in the absence of tractional retinal detachment affecting the macula has a beneficial effect on long term visual outcomes in PDR.

In the evolution of PDR, it would appear that partial separation with, vitreous adhesion to fibro vascular proliferative material is the precursor of catastrophic visual loss ⁶. This loss of
vision is caused by vitreous hemorrhage, tractional retinal detachment or a combination of these events. If vitrectomy were performed while visual acuity is at economically useful level, preservation of vision could be hoped for.

Patients and Method
150 eyes with PDR and poor visual acuity due to vitreous hemorrhage <3 months duration were subjected to pars plana vitrectomy and were followed up for a period of 5 years. The mean preoperative visual acuity was 3/60 standard 3- port pars plana 20/23 guage vitrectomy was performed. Peeling of epiretinal and subretinal membranes were performed as and when required. A pan retinal argon endolaser photocoagulation was performed intraoperatively in all the cases. The results obtained where compared to age, sex, and duration of DM matched cohort of 150 patients who underwent vitrectomy for complications of DR and in whom the duration of VH was > 6 months.

Results
The patients were of the age group from 45 to 70 years out of which 68 were males and 82 were females. All of them were non insulin dependent diabetics and had received prior laser photocoagulation either focal or pan retinal photocoagulation.

A detailed ophthalmic evaluation including BCVA, slit lamp biomicroscopy, applanation tonometry, dilated fundus examination, digital fluorescein angiography and OCT scan were carried out whenever possible. All patients underwent a B scan ultrasonogram. The patients were grouped into 2. Group 1 who underwent early pars plana vitrectomy and endolaser within 6 months of vitreous hemorrhage and Group 11 who underwent the same surgical procedure after 6 months from the onset of vitreous hemorrhage. All patients were evaluated postoperatively on day 1, day 15, day 30, 2nd month and 4 monthly there after.

The mean postoperative visual acuity improved from 3/60 to 6/18 by the end of 2 weeks (P=.002) and further improved to 6/9 (P= 0.008) at the end of the 5 year follow up period in 80 % of the patients; both of which were found to be statistically significant. 10 % of the above patients had active proliferative diabetic retinopathy, while progression of diabetic macular edema was observed in 15 % of the patients with respect to digital fluorescein angiogram and optical coherence tomography findings. 50 % of the above patients required cataract surgery while 3 % developed retinal detachment.

The mean preoperative visual acuity of gp ll pt ranged from PL + to 2/60. Pre op evaluation revealed presence of VH (80 %) TRD (15 %) pre macular hemorrhage (10 %) etc. The surgical procedure included PPV+MP+E/L PRP +/- PFCL inj and SO tamponade. The post operative visual acuity ranged from 6/60 to PL. Elevated IOP (25 %) ,NVG (15 %) ,phyysis (10 %) and recurrence of VH (20 %) were the post operative complications noticed in this group. 80 % in this group required cataract surgery within 5 years.

Discussion
The indications for and timing of pars plana vitrectomy for diabetic retinopathy continue to evolve, but they have not changed conceptually. The threshold for performing surgery in established indicated situations have generally been lowered. The lower threshold is attributable to improvements in both instruction and surgical techniques.

Historically the first indication for pars plana vitrectomy was severe non clearing diabetic vitreous hemorrhage.
Vitreous hemorrhage is probably the result of traction on the neovascular complexes from incomplete posterior vitreous detachment. As timely application of PRP has become more widespread, the incidence of profound visual loss from isolated dense vitreous hemorrhage has come down significantly. Less aggressive cases (those with less extensive neovascularisation and fibrovascular proliferation) respond more frequently to PRP than do cases with more aggressive retinopathy. Concurrently, the development of newer vitrectomy techniques and instrumentation allows successful outcomes even in more complex cases. As a result, the distribution of cases undergoing vitrectomy for non clearing vitreous hemorrhage has come down from 70% in 1977 to 2% in 1987.

Initially vitrectomy was deferred usually for 6 to 12 months to allow for spontaneous clearance of the vitreous hemorrhage, enabling delivery of stabilizing PRP. Since type 2 diabetics more commonly have slower progression of fibro vascular proliferation, the usual approach for patients with type 2 diabetes was to defer surgical intervention longer than for patients with type 1 diabetes. In contrast, vitrectomy is often considered earlier (within a few weeks) for type 1 diabetic patients especially if severe vitreous hemorrhage has shown no sign of spontaneous clearing. Currently, surgical intervention for non clearing vitreous hemorrhage is considered at an earlier time point in type 2 diabetics also. Several clinical features influence the recommended timing of vitrectomy for diabetic vitreous hemorrhage. Earlier surgical intervention is generally recommended when no previous laser treatment has been performed, when the fibro vascular proliferation complexes are more extensive and appear more vascular, when the fellow eye is blind. Surgical intervention is deferred, at least temporarily, when there is a complete PVD, when extensive prior PRP has been delivered, or when other medical conditions co exist. Patients with sustained hypertension or elevated levels of glycosylated hemoglobin should have prompt and appropriate treatment for these systemic conditions. Subsets of vitrectomy indications for vitreous hemorrhage are defined by co existing features. Ruberosis iridis in an eye with a recent vitreous hemorrhage, especially when no pan retinal photocoagulation has been applied constitutes an urgent indication for intervention.

An extensive subhyaloid macular hemorrhage constitutes another subset of surgical indication for media opacity. The confinement of blood in the sub hyaloid space indicates that the posterior hyaloid has not fully separated and remains a scaffold for progressive fibro vascular proliferation. Even though the haemorrhage may clear over several months, ongoing fibro vascular proliferation frequently establishes broad based zones on increasingly tight vitreoretinal adhesions. Because of the attendant poor visual prognosis after proliferation advances, surgical intervention should be considered relatively early in the course (within a few weeks after the onset).

A subretinal haemorrhage may occur spontaneously despite laser treatment and may severely limit final visual acuity if the macular is involved.

The degree of cataract may be difficult to assess in eyes with vitreous haemorrhage. Since lens opacities may be sufficient to impair not only the patients vision, but also the physicians ability to diagnose, monitor and apply retinal laser, cataract surgery may be performed either in combination with vitrectomy or as a 2 step procedure in these cases. Improved phacoemulsification techniques combined with intra operative photocoagulation account for improved outcomes reported with simultaneous Phacoemulsification - IOL implantation and vitrectomy in selected cases.

Severe recurrent vitreous hemorrhage (either before or after vitrectomy) may induce a secondary glaucoma through a ghost cell mechanism. Most cases with increased intraocular pressure caused by vitreous hemorrhage respond to medical therapy. However selected cases with poorly controlled intraocular pressure despite maximum medical treatment may respond to repeat vitrectomy by relieving the outflow blockage caused by red blood cells. In many cases, however post operative vitreous hemorrhage severe enough to persist beyond about 6 weeks is a manifestation of re proliferation, retinal break formation, or other severe complications that require reoperation.

The results of vitrectomy for non clearing diabetic vitreous haemorrhage have been reported and reviewed extensively and vision improved in 70-80% of patients and a final visual acuity of 20/200 or better in 50-60% has been reported. The Diabetic Retinopathy Vitrectomy Study demonstrated that early vitrectomy (1-4 months after the onset of vitreous haemorrhage) for type 1 diabetics yields visual acuity outcomes of 20/40 or better at 2 years in 36% of this subgroup compared to only 12% with conventional management (p=0.001). Our study for vitreous haemorrhage in patients with type II diabetes can lead to long term improvement in visual acuity and stabilization of retinopathy after early vitrectomy.

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ABSTRACT

Objective: to analyze the various clinical presentations, prognostic factors, diagnostic methods, treatment modalities, outcome of treatment and the complications of treatment of the cases of orbital lymphoma diagnosed in a tertiary eye care centre in Kerala and were treated in cancer treatment centre from January 2006-December 2010. It was a retrospective non comparative case series of patients diagnosed histologically as orbital lymphoma at this centre and received treatment in a cancer centre. Results: Of the 32 cases which were diagnosed as orbital lymphoma 26 cases received treatment in a cancer centre. Mean age of the patients who presented with orbital lymphoma was 52.8 ±23.4 yrs with a range of 29-83 yrs males to female ration was 2.55:1. Major presenting complaint was proptosis in 68% and restricted ocular motility in 56%. A visible or palpable mass was present in 93% of the patients of which 50 % had a subconjunctival mass. All the cases were diagnosed by incision biopsy. The dose of radiation received by each patient varied between 24 to 36 Gy and the complications post radiotherapy were dry eye, cataract and punctual stenosis. Discussion: in our series, visible mass and proptosis were the major presenting complaints which are similar to the other reported data. All the patients who were treated received radiation therapy for the local control of the disease and all the patients had good local control of the disease with radiation therapy. Conclusion: the role of an ophthalmologist in the management of orbital lymphoma is not limited to the diagnosis of the disease by biopsy but also extends to the diagnosis and treatment of complications of treatment. Radiation remains the primary therapy for the local control of the disease.

INTRODUCTION

Lymphoproliferative tumors are the most common primary orbital neoplasms in adults yet constitute only approximately 2% of all lymphomas.1 Lymphoid tumors constitute approximately 10% of all orbital tumors. A majority of orbital lymphomas are of the non-Hodgkin’s type and are seen mostly in adults during the fifth and the seventh decades of life.

The commonest manifestation of the disease is a slowly growing orbital mass that can be either asymptomatic, or, depending on the location of the tumor, associated with proptosis, ptosis, ocular dysmotility, periorbital or subconjunctival swelling, blurring of vision and chemosis. The most frequent histology is extranodal marginal B-cell lymphoma of mucosa-associated lymphoid tissue (MALT).2 Usually therapeutic management of POL consists of radiation treatment using low or moderate doses in the range of 25-36 Gy, can obtain 95-100% of local control.

The variations in radiation effect on the eye are dependent not only on tissue sensitivities but also on the methods of radiation delivery. The common complications reported are dry eye, cataract and radiation retinopathy5.

Though there are many numbers of published data on orbital lymphoma from all over the world and a few published reports from different parts of our country, comprehensive data from our part of the country is lacking. With this in mind, we tried to analyze our experience with orbital lymphoma.

AIM

To study the demographic profile, Clinical presentations, the prognostic factors, treatment modalities, outcome and Complications of treatment.

MATERIALS AND METHODS

Retrospective non comparative case series of all the patients who were diagnosed histologically as orbital lymphoma and were referred for staging work up and radiation therapy.

Inclusion criteria

who were diagnosed as orbital lymphoma any histological type by biopsy in this centre from January 2006 to December 2010 and were referred to oncology center for staging work up and radiation therapy.

Exclusion criteria

The patients who had a benign or malignant swelling histopathologically other than lymphoma

The medical records of all the patients who satisfied the inclusion criteria were retrieved and the data was collected regarding the details of clinical presentation and treatment.

• Identification details like name, age, sex, address and contact details were recorded
• Details of clinical presentation including symptoms,
associated systemic diseases, details of previous treatment taken
• Clinical examination findings: best corrected visual acuity, proptosis, ptosis, subconjunctival or peri orbital mass, limitation of ocular motility
• Results of investigations: CT scan orbit, serum lactate dehydrogenase (LDH)
• Details of the surgical procedure for biopsy, either incision biopsy excision biopsy or orbitotomy and the histological diagnosis
During the regular follow up of the patients at this centre after the radiation treatment is over, the data is recorded in terms of
• Symptoms of recurrence or residual disease: proptosis, mass around the eye
• Symptoms of complications of treatment: dryness, burning sensation, defective vision, history of topical medications or cataract surgery or any other surgery done
• Clinical examination details were recorded: acute radiation toxicity, Schirmer test results, presence or absence of lenticular opacity, presence or absence of retinopathy, any other complications of surgery or radiation such as ptosis, punctal stenosis or conjunctival scarring.

A diagnosis of dry eye was based on the symptomatology and clinical examination findings. A patient was considered to be having cataract progression if there is a history of cataract surgery or if there is a lenticular opacity on slit lamp examination.

The following details of the staging work up and radiation treatment were collected from the patients from the medical records (discharge summary, medical reports) available with them.

• Stage of the disease
• Histology
• Immunohistochemistry work up
• Total dose of radiation and number of fractions
• Any side effects during or after the treatment

The data thus collected and recorded were analysed statistically.

Statistical analysis
Categorical data was expressed as percentage. The statistical tests used were Spearman’s co-efficient of rank correlation, Cox proportional-hazards regression and Kaplan-Meier survival analysis. Only p-values lower than 0.05 were considered significant.

RESULTS
During the period from January 2006 to December 2010, 32 patients were diagnosed histologically as orbital lymphoma from Little Flower Hospital and Research Centre Angamaly and referred for immunohistochemistry studies, staging work up and treatment.

Demographic profile
The patient characteristics are given in Table 1.

Follow up data
Of the 32 patients who were histologically diagnosed as orbital lymphoma and referred for further treatment, treatment details were available from 28 patients. 3 cases were lost to follow up and one patient died during the study period due to HIV-AIDS complex. The mean follow up period was 13.59 months (range 1-59) (SD 13.44). 26 patients received treatment from any one cancer centre in Kerala either local radiation therapy or systemic chemotherapy. One

<table>
<thead>
<tr>
<th>Males/females</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>23/9</td>
<td></td>
</tr>
<tr>
<td>Median age</td>
<td>52 (range 29-83)</td>
</tr>
<tr>
<td>Mean follow up</td>
<td>13.59 (range 1-59)</td>
</tr>
<tr>
<td>Mean duration of symptoms before diagnosis</td>
<td>17.73 months (range 2 weeks-10 yrs)</td>
</tr>
<tr>
<td>Localization</td>
<td></td>
</tr>
<tr>
<td>Conjunctiva</td>
<td>11(34%)</td>
</tr>
<tr>
<td>Orbital soft tissue</td>
<td>17(53%)</td>
</tr>
<tr>
<td>Lacrimal gland</td>
<td>4(13%)</td>
</tr>
<tr>
<td>Laterality</td>
<td></td>
</tr>
<tr>
<td>Right eye</td>
<td>10(31.25%)</td>
</tr>
<tr>
<td>Left eye</td>
<td>19(59.37%)</td>
</tr>
<tr>
<td>Both eyes</td>
<td>3(9.3%)</td>
</tr>
</tbody>
</table>

Table 1: Table showing the patient and the tumor
Aparna C Varghese - Ocular Lymphoma

Clinical presentation
The frequency distribution of common clinical features are given in Fig 1.

Figure 1: bar diagram showing the frequency distribution of the different clinical presentations of orbital lymphoma.

H/o previous treatment with systemic steroids was present in 12 patients with improvement of the symptoms, but recurrence on withdrawal of steroids.

History of previous surgery either complete excision or incision biopsy was present in 3 patients which was not followed by radiotherapy. There was history of treatment with alternative medicine in one patient.

Most common site was the orbital soft tissue followed by the subconjunctival tissue and the lacrimal gland.

Visual acuity at presentation
The mean logMAR visual acuity at presentation was 0.84 ±0.32 (0.7237 to 0.9613 95% CI for the mean). The mean logMAR visual acuity at last follow up was 0.8569 ± 0.2695 (0.7597 to 0.9540 95% CI for the mean). There is no statistically significant change in visual acuity during the study period (Wilcoxon matched pairs signed rank test (P = 0.8457).

Associated diseases
One patient had HIV-AIDS. 3 patients had Diabetes mellitus, 7 patients had systemic hypertension and h/o coronary artery disease was present in 3 patients. All the patients had normal thyroid function tests.

Imaging studies: CT scan was available in 28 patients. The CT scan finding was a homogenous moderately enhancing mass isodense to muscles with moulding to the globe, optic nerve and bone. 62% of the cases showed an extracanal mass lesion and 4 cases had lacrimal gland enlargement and 2 had EOM enlargement.

Surgical procedure: The surgical procedure was selected depending on the site and accessibility of the lesion. 2 cases had lateral orbitotomy for lacrimal gland mass and in all cases the histological diagnosis was achieved by incision biopsy.

Histology
The most common histology in our series was Extranodal marginal zone lymphoma of mucosa associated lymphoid tissue (MALT) 53% and 3 cases (11%) had an aggressive phenotype such as Diffuse large B-cell lymphoma (DLBCL). The distribution of histology of orbital lymphoma in our series is represented in Fig 2.

Staging of the disease: None of the patients whose follow up data was available had any other system involvement any systemic symptoms like fever. Thus all the patients were staged as Stage IA according to Ann Arbor classification. Of the 3 patients who had bilateral disease 2 patients were on regular follow-up till the 2010 June and none of them developed any systemic disease during this 2 yrs period.

Results of treatment
26 patients in our series from whom the treatment was available received radiation treatment at a dose of 24-46 Gy with 1.8-2 Gy per fraction. All patients attained local control of the disease after radiotherapy. The disease free survival at 5 yrs was 78.3% (SE 0.109). The Kaplan Meier survival curves for disease free survival is as shown in fig 3.

The overall survival at 5 yrs was 96.9%. All patients had improvement of their presenting symptoms after radiation. All patients with proptosis had symptomatic relief by the end of the course of radiation. The patients with visible masses on the eyelids and conjunctiva had complete resolution.

Recurrence of the disease
3 cases (11.5%) had recurrence in the ipsilateral orbit and one case (3.8%) had recurrence in the contralateral orbit.
Patient age, sex, duration of complaints, bilaterality, anatomic localization of the lesion, intracanal location of the lesion in CT scan did not influence failure-free survival with Cox multivariate regression analysis of risk factors.

The major complications that were reported in our study series are depicted in figure 4 below.

All the patients in our study group belonged to the Stage IAE and there was no systemic involvement in any of the cases. This is comparable to the 5% incidence of systemic involvement reported by a recent large series of 95 cases.8

96% of the patients in our study group received radiation therapy at a total dose of 24-36 Gy with 1.8-2 Gy per fraction either by anterior field technique or multiple oblique fields which similar to the radiation dose reported by other studies.16,17

The overall survival and disease free survival in our series was 96.9% and 78.3% respectively at 5 yrs with 11% of ipsilateral recurrence and 3.8% of contralateral recurrence. This is similar to the recurrence rates reported by many investigators.19, 20 In our study age >60 yrs, female sex, bilaterality, conjunctival localization or intracanal location did not affect the failure free survival in orbital lymphoma. This is in contrast to the rare cancer network study by Martinet ET al19 but similar to the results reported by many other investigators.7, 20

The incidence of complications of radiation therapy reported in our study are similar to the data reported by other studies also.7

Conclusions
According to our study, in our part of the world, lymphomas of the orbit are uncommon, indolent diseases and may involve any site in the orbit. Full staging work up is mandatory for proper management. Radiotherapy is an excellent treatment modality for primary orbital lymphoma. Radiation doses of
30 to 35 Gy are adequate for local control. Surgery helps in providing the material for the accurate tissue diagnosis. Radiation toxicity can be minimized by meticulous planning and proper dose prescription. The role of the ophthalmologist does not end at the diagnosis of the disease and prompt referral but continues to the detection and timely treatment of the complications of radiation therapy.

References

Dr Aparna Cessai completed her training from Little Flower eye hospital and is now consultant Ophthalmologist working at Vasan eyecare hospital
Iatrogenic Peripheral Retinal Breaks in 20-G Pars Plana Vitrectomy

ABSTRACT
Aim-To determine frequency and risk factors for iatrogenic peripheral breaks in eyes undergoing 20-G three port pars plana vitrectomy (3PPV).

Materials and Methods-Single-centre, retrospective, interventional case series. A total of 56 patients undergoing PPV from January 1, 2009 to January 1, 2011. Main outcome measures were frequency, anatomic location and risk factors associated with iatrogenic peripheral retinal breaks and rate of post-operative rhegmatogenous retinal detachment.

Exclusion criteria included pre-existing retinal breaks, rhegmatogenous retinal detachment, vitrectomized eyes and iatrogenic breaks posterior to the equator.

Results-Iatrogenic peripheral retinal breaks occurred in 9 of 56 eyes (16%). Three of 56 cases (5.3%) experienced postoperative rhegmatogenous retinal detachment caused by undetected or new peripheral retinal breaks. Breaks were most common during surgery for posterior dislocated nucleus (40%), macular surgeries (15%) and surgery for vitreous hemorrhage and tractional retinal detachment (13.3%). Breaks were more common in the superior retina (77.7%) with majority occurring in the 10'0 clock and 2'0 clock positions. Difficult induction of posterior vitreous detachment and a near total vitrectomy was associated with development of iatrogenic peripheral breaks.

Conclusion-Iatrogenic peripheral retinal breaks is a definite risk factor for postoperative retinal detachment. Difficult induction of posterior vitreous detachment and a near total vitrectomy were risk factors for development of iatrogenic peripheral breaks.

Introduction
Indications of modern pars plana vitrectomy include macular surgery (for macular hole, epiretinal membrane, vitreomacular traction syndrome, or macular edema) and vitrectomy for proliferative diabetic retinopathy or retained lens matter after complicated cataract surgery. Despite advances in vitreoretinal surgery secondary retinal detachment caused by iatrogenic peripheral retinal breaks remains the most common sight threatening complication of this surgery. (1–4) Many iatrogenic retinal tears are associated with traction at sclerotomy entry sites, occurring intraoperatively as the result of insertion and removal of instruments that engage the vitreous base or postoperatively as the result of fibrovascular healing or contraction of incarcerated vitreous. (5)
Surgical indication Stratified by Retinal Break Formation (Table 1)

<table>
<thead>
<tr>
<th>Indication/Diagnosis</th>
<th>Iatrogenic Retinal Breaks</th>
<th>%</th>
<th>No Retinal Breaks</th>
<th>%</th>
<th>RD</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRD</td>
<td>2</td>
<td>6.6</td>
<td>12</td>
<td>93.4</td>
<td>1 (7.1%)</td>
</tr>
<tr>
<td>VH</td>
<td>2</td>
<td>12.5</td>
<td>14</td>
<td>87.5</td>
<td>1(6.25%)</td>
</tr>
<tr>
<td>Nucleus drop</td>
<td>2</td>
<td>40</td>
<td>3</td>
<td>60</td>
<td>1(20%)</td>
</tr>
<tr>
<td>Macular hole</td>
<td>3</td>
<td>15</td>
<td>17</td>
<td>85</td>
<td>1(5%)</td>
</tr>
<tr>
<td>Dislocated IOL</td>
<td>0</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TRD- Tractional retinal detachment, VH – Vitreous hemorrhage.

Vitreous hemorrhage vitrectomy-Analysis (Table 2)

<table>
<thead>
<tr>
<th>Vitreous hemorrhage vitrectomy</th>
<th>Number</th>
<th>Iatrogenic Retinal Breaks</th>
<th>%</th>
<th>No Retinal Breaks</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Near total (1)</td>
<td>10</td>
<td>2</td>
<td>20</td>
<td>8</td>
<td>80</td>
</tr>
<tr>
<td>Others (2)</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(1) Near total vitrectomy, (2) Subtotal vitrectomy.

Phakic status and PVD (Table 3)

<table>
<thead>
<tr>
<th></th>
<th>PVD present</th>
<th>PVD absent</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phakic</td>
<td>10(29%)</td>
<td>16</td>
<td>26</td>
</tr>
<tr>
<td>Pseudophakic</td>
<td>24(71%)</td>
<td>6</td>
<td>30</td>
</tr>
</tbody>
</table>

Demographic and Clinical Characteristics Stratified by Retinal Break Formation (Table 4)

<table>
<thead>
<tr>
<th></th>
<th>IRB</th>
<th>%</th>
<th>NRB</th>
<th>%</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>4</td>
<td>44.5</td>
<td>24</td>
<td>51</td>
<td>0.716</td>
</tr>
<tr>
<td>female</td>
<td>5</td>
<td>55.5</td>
<td>23</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>Eye</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>5</td>
<td>55.5</td>
<td>25</td>
<td>53.2</td>
<td>0.896</td>
</tr>
<tr>
<td>Left</td>
<td>4</td>
<td>44.5</td>
<td>22</td>
<td>46.8</td>
<td></td>
</tr>
<tr>
<td>PVD</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>preexisting</td>
<td>1</td>
<td>11</td>
<td>33</td>
<td>70.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>induced</td>
<td>8</td>
<td>82</td>
<td>14</td>
<td>29.8</td>
<td></td>
</tr>
<tr>
<td>Lens status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phakic</td>
<td>6</td>
<td>66.6</td>
<td>32</td>
<td>68</td>
<td>0.933</td>
</tr>
<tr>
<td>Pseudophakic</td>
<td>3</td>
<td>33.4</td>
<td>15</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Total eyes</td>
<td>9</td>
<td>100</td>
<td>47</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

IRB-iatrogenic retinal breaks, NRB- No retinal breaks
Results
Iatrogenic peripheral retinal breaks occurred in 9 of 56 eyes (16%) which satisfied the eligibility criteria during the study period. Overall, the mean age was 58.3±16.9 years. Average follow-up after vitrectomy was 6 months.

Indications for Vitrectomy and Peripheral Retinal Breaks
Table 1 stratifies indication for vitrectomy by the presence or absence of iatrogenic peripheral retinal breaks. The most common indication for surgery was macular hole repair (35.7%) followed by vitreous hemorrhage clearance (28.5%). Breaks were most common during surgery for posterior dislocated nucleus (40%), macular surgeries (15%) and surgery for vitreous hemorrhage and tractional retinal detachment (13.3%). There was no correlation between stage of macular hole and break formation.

Risk Factors for Iatrogenic Peripheral Retinal Breaks
Table 4 summarizes patient demographics, laterality, and preoperative PVD status and lens status in those with peripheral iatrogenic breaks compared with those without. Posterior vitreous detachment was preexisting in 60.7% of cases; 53.5% were pseudophakic. Eyes with a preexisting PVD were more likely to be pseudophakic (71%) than eyes without PVD (Table 3). No difference in gender or lens status was found between those developing and not developing iatrogenic retinal breaks. A statistically significant difference in preoperative PVD status was found between eyes that developed breaks and eyes that did not. In cases of vitreous hemorrhage a near total vitrectomy was associated with a 20% chance of developing peripheral retina breaks.

Number and Distribution of Iatrogenic Peripheral Retinal Breaks
Iatrogenic peripheral retinal breaks occurred in 9 of 56 eyes (16%). Overall, iatrogenic retinal breaks occurred more frequently in the superior retina (77.7%), with 66.6% of the breaks occurring in the 2 and 10 o’clock positions, coincident with superonasal and superotemporal sclerotomy sites. No association between a surgeon’s dominant right hand and location of retinal breaks was observed.

Iatrogenic Break-related Retinal Detachment
After vitrectomy, 3 of 56 eyes (5.3%) developed postoperative rhegmatogenous retinal detachment as a result of new or missed iatrogenic breaks. Two of these cases had peripheral retinal breaks identified and treated at the time of primary vitrectomy and in the remaining one no break was detected during the search at the conclusion of primary vitrectomy. Surgical induction of PVD was performed in all 3 cases. Table 1 shows the distribution and incidence of retinal detachment in our series.

Discussion
Secondary retinal detachment caused by unidentified iatrogenic peripheral retinal breaks remains an important sight threatening complication of 3PPV. Breaks commonly occur intraoperatively at the posterior border of the vitreous base as the result of traction from instrumentation. In addition, peripheral retinal tears may occur postoperatively in the region of sclerotomy entry sites because of contraction of incarcerated vitreous.

Frequency of Iatrogenic Retinal Breaks and Retinal Detachment after Vitrectomy
In eyes undergoing 3PPV for proliferative diabetic retinopathy, tractional retinal detachment, rhegmatogenous retinal detachment, or penetrating eye injury, entry site breaks have been reported to occur in 3.1% (14/447) intraoperatively or within 10 days of surgery. For macular hole surgery, the prevalence of iatrogenic breaks has been reported between 0% and 14.6% (7,8,10,14) with postoperative retinal detachment occurring in 1.1% to 14%. In a series of 244 patients undergoing 3PPV for macular hole, epiretinal membrane, or diabetic macular edema, Wimpissinger and Binder (13) reported no intraoperative entry site breaks, but a postoperative sclerotomy-associated retinal detachment rate of 4.5%. This data compares well with our series. However, our data showed a higher incidence of retinal detachment and peripheral breaks in cases with nucleus drop. This could be because of the compromised retinal status due to the initial surgery or trauma and the tractional forces in play during the procedure. Wimpissinger and Binder (13) who, in their series of 244 eyes, found only 1 phakic patient among 11 who developed entry site break-related rhegmatogenous retinal detachment. In our series we did not find any difference in relation to the phakic status of the eye. Near total vitrectomy especially for vitreous hemorrhage could lead to small breaks missed during initial surgery and lead to retinal detachment later. Hence a thorough peripheral search and cryotherapy is absolutely in such cases.

Location of Iatrogenic Peripheral Retinal Breaks
This study found that iatrogenic peripheral retinal breaks were more common in superior quadrants, with apparent clustering around sclerotomies. This is in agreement with a mixed case series by Moore et al. (9) who reported that the majority of breaks (69%) occurred in the superior retina. However, in a more recent series of macular hole and epiretinal membrane surgery, no difference in distribution of breaks in superior compared with inferior retina was found. Tan et al (11) also reported the induction of a PVD to be associated with a higher rate of breaks elsewhere as opposed
to sclerotomy associated breaks. In terms of lateralization of breaks, previous findings in diabetic vitrectomy have demonstrated that most peripheral breaks are sclerotomy related, associated with the surgeon’s dominant hand, (4) and Moore et al (9) found that only large breaks (1 clock hour) were associated with the surgeon’s dominant right-hand sclerotomy. We could not make a definite conclusion about this in our series. 

**Surgical Induction of Posterior Vitreous Detachment and Peripheral Retinal Breaks**

In a series of 634 eyes, Guillaubey et al (14) identified a higher incidence of iatrogenic breaks and retinal detachment after macular hole surgery compared with epiretinal membrane cases, attributing this difference largely to the act of surgically detaching the posterior hyaloid. However, in our series a higher frequency of breaks was associated with surgical induction of PVD (82%, P < 0.001) compared with cases with preexisting PVD. All cases of postoperative retinal detachment had undergone surgical induction of PVD during the initial surgery. This trend was seen distributed among all the indications for vitrectomy in our study. Preexisting PVD was more common in pseudophakes (71%) in this series.

**Preventing Iatrogenic Peripheral Retinal Breaks**

Our findings highlight that iatrogenic peripheral break formation continues to be an inevitable risk of vitrectomy and varying with indication. The mainstay of reducing post-vitrectomy retinal detachment relies on avoiding, detecting, and treating breaks during surgery with an emphasis on careful search of the peripheral retina, particularly at sclerotomy entry sites before completion of surgery and especially after PVD induction. Other precautions we recommend include minimizing instrumentation and the number of instrument changes, ensuring good vitreous clearance at sclerotomy sites, avoiding vitreous incarceration and cryotherapy behind sclerotomy sites. It is known that multiple use of disposable instruments, such as the vitrectomy cutter, may be associated with blunting, vitreoretinal traction, and therefore increased occurrence of retinal breaks. Microincision vitreoretinal surgery using 23- or 25-gauge cannulated systems may be advantageous in reducing the incidence of sclerotomy-associated breaks. (20, 21)

However, a high rate of peripheral break formation (15.8%) was recently reported in 177 eyes undergoing 25-gauge vitrectomy for macular hole or epiretinal membrane, (11) suggesting that even when traction from instrumentation is minimized, a major determinant of iatrogenic retinal tears is surgical separation of the posterior hyaloid from retina. 

**Conclusion**

In conclusion, iatrogenic peripheral retinal breaks occur with significant frequency during vitrectomy, with a wide variation by indication. Difficult induction of posterior vitreous detachment and a near total vitrectomy, in vitreous hemorrhage was associated with development of iatrogenic peripheral breaks.

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of 77 consecutive cases of 23-gauge transconjunctival
vitrectomy surgery for posterior segment disease.

Dr Ashok Nataraj finished his MS from KMC Mangalore and then did his retinal training from Sankara Nethralaya Chennai.
Presently he is head of retinal services at Tony’s superspeciality Eye hospital, Aluva.
Abstract

Purpose: To study the anatomical and functional outcome of Therapeutic keratoplasty in fungal keratitis.

Methods: A prospective non comparative interventional study was conducted from January 2008 to June 2009 among 34 patients with fungal keratitis who underwent Therapeutic keratoplasty. All the cases were microbiologically and histopathologically proven as fungal. Patients included in this study had a minimum follow up of 6 months and maximum of 1 year. The results were evaluated for each of the following criteria; anatomical integrity, graft survival, best corrected visual acuity, and complications.

Results: Anatomical integrity was achieved in 84% of patients. Graft clarity was achieved in 56%. BCVA of ≥ 6/60 was achieved in 30%. Major postoperative complications included graft failure with rejection (n=8) noted in larger grafts (> 10.5 mm), high IOP (n = 3), and recurrence of infection (n = 3).

Conclusion: Therapeutic PK is effective in the management of severe refractory fungal keratitis achieving high cure rates. Approximately half of our patients maintained a clear compact graft at the last visit.

Key words: Therapeutic Keratoplasty, fungal keratitis, graft survival, anatomical integrity

Introduction

The term ‘Therapeutic Keratoplasty’ is used for a surgical procedure performed either for restoration of structural integrity of the eye or to resolve an infectious and inflammatory keratitis which is refractory to conventional medical treatment. Because of the recent development of more potent but less toxic antifungal agents, major advances have been made in the treatment of fungal infections. Nevertheless, refractory fungal keratitis still poses a therapeutic challenge as it may progress to corneal perforation and fungal endophthalmitis. Without prompt and effective management, accompanied inflammation may also result in extensive anterior or posterior synechiae, secondary intractable glaucoma, and even extrusion of intraocular contents. To arrest the progression of infection, preserve the globe integrity and avoiding disastrous complications, therapeutic penetrating keratoplasty (TPK) has been advocated for severe fungal keratitis.

The procedure can be a full thickness penetrating keratoplasty or a partial thickness lamellar keratoplasty. As the surgery is most often performed on an inflamed eye, the postoperative course and chances of graft survival are different from an optical keratoplasty. Hence the purpose of our study was to evaluate the anatomic and functional outcome of Therapeutic keratoplasty in fungal keratitis.

Materials and Methods

A prospective interventional study was performed among 34 patients with fungal corneal ulcer who underwent Therapeutic Keratoplasty from January 2008 to June 2009 in the cornea clinic at Little Flower Hospital.

The included patients were cases of advanced keratitis unresponsive to conservative management which were preoperatively proven microbiologically, cases of imminent corneal perforation and perforated corneal ulcers, who underwent Therapeutic PKP and were subsequently histopathologically proven as fungal keratitis. In the study group there were 21 eyes (61.7%) with an ulcer refractory to medical treatment, 6 eyes with impending perforation (25%) and 7 perforated eyes (20.6%). Patients included had a minimum follow up of 6 months and a maximum of one year. Patients with associated posterior segment pathology were excluded from the study.

A detailed ocular examination was done preoperatively including vision, slit lamp examination, and serial documentation regarding the extent of involvement particularly in relation to the limbus, evidence of scarring, vascularization, anterior segment inflammation and corneal perforation. A detailed fundus examination was also attempted, if possible and in large non perforated ulcers B–scan ultrasonography was done Corneal scrapings for smears and culture were obtained under slit lamp with No.15 Bard Parker blade from the edges and base of the ulcer.

Based on the microbiological results 27 patients were excluded from the study. Seven patients had perforation at presentation. Oral Ketoconazole 200 mg twice daily was started for patients with deep ulcers, larger ulcers (more than 5 mm) and also in ulcers with perforation. Three of the seven perforated eyes...
were treated with tissue adhesive and bandage contact lens initially as the perforation was less than 2mm. All these three eyes subsequently showed progression of infiltration and required therapeutic keratoplasty.

The donor corneas were obtained from our own eye bank. Corneas were stored at 4°C in McCarey-Kaufman media. The mean donor age was 62.9 years (range 37-82) and mean preservation time was 47.14 hours (range 2-92). The graft size ranged from 7.5-11 mm (mean 8.8). The quality of donor tissue was assessed by slit lamp examination and only good quality donor tissues were taken for transplantation. All the surgeries were done under local anaesthesia by a single surgeon.

All patients were given 250 ml of 20% mannitol intravenously preoperatively. Self retaining lid speculum was used. In case of large ulcers that reached up to the limbus peritomy was done and hemostasis was achieved with wet field cautery. After a thorough AC wash corneal infiltrate was measured using a caliper. Corneal trephine 1mm larger in diameter than the size of infiltration was selected. 80% of depth of the cornea was trephined followed by a guarded entry into the anterior chamber with a number 11 surgical blade. Trephination of the recipient bed was technically difficult especially in cases with corneal necrosis, gross thinning and perforation. Anterior chamber entry was followed by injection of viscoelastic material to deepen the peripheral anterior chamber and release the anterior synechiae. Universal corneal scissors were used to complete the excision. After removal of the diseased cornea, hypopyon and fibrinous material were carefully removed and the recipient bed was prepared. Peripheral iridectomy was done.

In all cases the corneal specimens were subjected to both microbiologic and histopathologic evaluation. The donor cornea was trephined 0.5mm larger than recipient size and was transferred using a spatula. The donor recipient junction was sutured with interrupted sutures using 10-0 Nylon. At the end of the surgery the anterior chamber was deepened by BSS. The crystalline lens was inadvertently removed in one eye during the removal of fibrin; all others were left untouched even though some opacity was noted during Therapeutic PKP.

Postoperatively all patients were maintained on T.Ketoconazole 200mg bd, topical Natamycin 5% QID, and Atropine 1% e/d TDS. Anti glaucoma medications T.Acetozolamide 250mg QID and topical Timolol maleate 0.5% bd were added as required. Wound leak, epithelial defect and residual infection were assessed in the postoperative period. Systemic and topical antifungals were continued for 14 days. After 2 weeks topical steroids [Prednisolone acetate 1%] were started. Thereafter patients were followed up weekly for the first month, every 2 weeks for 2 months, monthly for a minimum of 6 months and at different intervals after that.

The factors analyzed included anatomical results, graft survival, best corrected visual acuity and complications. Anatomical success rate was defined as preservation of the globe and thus avoidance of evisceration. Graft survival was defined as the presence of a clear and functional graft at the last follow up visit. Mean follow up was 7.4 months.

Comparison between pre op and post op vision was done by paired sample t-test. Comparison of graft survival between large and small graft size was done by Kaplan Meyer survival analysis. P value was calculated to know the significance between 2 groups. Comparison of outcome between early and delayed surgery was also done by Kaplan Meyer survival analysis. P value less than 0.05 was considered as significant.

Results
34 eyes of 34 patients who underwent therapeutic penetrating keratoplasty were enrolled in our study. 58.8% were males and 41.1% were females with average age of 53.7 years. [27-90 years]. Left eye was found to be more commonly involved [76.47%]. A history of recent corneal injury was obtained in 16 (47%) patients.

Pie Diagram showing the predisposing factors

Vision at Presentation

Figure 1.

Figure 2.
85% of patients showed early features of scarring before surgery.

**Anatomical Integrity**

31 of 34 eyes (91.1%) showed successful eradication of infection and their globe integrity was preserved.

One of the patients who had re-infection (case no.7) initially presented with a deep stromal infiltrate with limbal involvement and hypopyon. 11 mm was the maximum trephine available. Residual infiltrate was there in the periphery. Repeat PK was done after 2 weeks. But she presented again with graft infiltrate. Graft melted with iris prolapse and the eye went into phthisis.

Second patient (case no.8) had presented with a deep stromal total corneal infiltrate with stromal lysis and secondary glaucoma. Graft size used was 10.5mm. Developed graft infiltrate postoperatively and eye went into phthisis.

Third patient (case no 6) who developed recurrence resolved with medical management.

**Graft Survival**

Out of thirty two eyes, 12 grafts remained clear till the last follow up. Twenty had graft rejection (62%) and developed vascularized corneal opacity. Graft size (>9mm) was used in 11 patients (32.3%) and less than 9 mm was used in 23 patients. We achieved a 74% rate of graft clarity when the graft size was 9mm or less, [n=23] whereas after including the larger sized graft, [n=11] the percentage of graft clarity was reduced to 61%.

Kaplan Meyer Survival analysis curve of patients with large (>9mm) and small (<9mm) showed a significant difference in survival rate (p value = 0.034) between the two groups. Postoperatively, the final visual acuity was 6/24 or better in 13 eyes (38%), 1/60 or better in 10 eyes (29%), less than 1/60 in 11 patients (32%). The following graph compares the final visual acuities.

**Postoperative Complications**

The only intraoperative complication was extrusion of the crystalline lens in one patient. Commonest postoperative complication noted was elevated IOP. Thirteen
of 34 eyes (38.2%) developed elevated intraocular pressure post operatively. 12 out of 13 cases were treated with antiglaucoma medications. One case which was refractory to topical medications was successfully managed with trabeculectomy

DISCUSSION

This study, evaluated the outcome of Therapeutic Keratoplasty in two years duration in 34 eyes of 34 patients in a tertiary eye care hospital.

We found that the proportion between male and female patients who underwent keratoplasty was nearly equal, 58.8% and 41.1% respectively. Their ages ranged from 27-90 years and their average age was 53.7 years. Corneal ulcer occurred most frequently in the age group of 40-60 years (58.8%). In the study conducted by Aravind eye hospital, Madhurai most of the patients (66.4%) with fungal keratitis were between 21 and 50 years.\(^2\)

A history of recent corneal injury was obtained in 16 (47%) patients. Study conducted by Disha eye hospital, West Bengal also found that ocular trauma was the most common predisposing factor in 82.9% followed by use of topical corticosteroids in 19.28%.\(^3\)

Left eye was found to be more commonly involved. The time of presentation in this study ranged from 0-30 days, mean time interval was (7.2 days). 31 among 34 patients (91.2%) presented with a vision less than 1/60. 4 patients (11.7%) had only PL+ vision. 7 patients had perforation, 6 had imminent corneal perforation and 4 patients had a large infiltrate of more than 7mm. Commonest indication for surgery was non resolving ulcer.

16 eyes were diagnosed based on the smear reports revealing fungal Hyphae in KOH wet mount. Culture showed Fusarium in 8 eyes and Aspergillus in 10 eyes. Aspergillus was the commonest fungus isolated.

Thirty one of 34 eyes (91.1%) showed successful eradication of corneal fungal infection without recurrence, and their globe integrity was preserved without intractable complications after surgery. Thirteen of 34 eyes (38.2%) developed elevated intraocular pressure after surgery even though corneal fungal infection was eradicated. Late onset secondary glaucoma results from extensive peripheral anterior synchiae and may lead to graft failure. The incidence of secondary glaucoma has been reported as 50% in fungal keratitis by Singh G et al.\(^4\)

Three patients had recurrence of infiltrate (8.8%). It should be considered as an emergency and should be treated intensively with topical fortified drugs and a repeat surgery, if needed. Polack et al reported a graft infiltrate recurrence rate of 7.3-10% in keratomycosis.\(^5\)

Cataract developed in 23.5% of patients (8 among 34 patients) following surgery. The cataract may be surgery induced or steroid induced. Panda et al reported an incidence of 3-50% cataract following keratoplasty.\(^6\)

Graft rejection is a common cause of graft failure and occurs in 14.6% to 52.1% of cases after therapeutic penetrating keratoplasty.\(^7\) Our study showed an incidence of 23.5%. The timely removal of loose and vascularized sutures and adequate control of inflammation with use of steroids is important in the prevention of graft rejection. Early diagnosis and treatment can reverse graft rejection in most cases.

Postoperatively, the final visual acuity was 6/24 or better in 13 eyes (38%), 1/60 or better in 10 eyes (29%), less than 1/60 in 11 patients (32%). The clarity of graft depends on the size of the graft. Larger grafts give a worse prognosis because of more chances of immunologic graft rejections with vascularization, development of posterior synchiae and secondary glaucoma. We achieved a 74% rate of clear grafts when the graft size was 9mm or less, whereas after including the larger sized graft, the percentage of clear grafts was reduced to 61%. Du and co-workers reported a clarity rate of 89% with a graft size of 7mm or less, compared to 21% clarity rate in cases in which grafts were 8 mm or larger.\(^8\) Killingsworth and co-workers achieved clear graft in 75% of fungal keratitis when smaller grafts were used compared to 33% when larger grafts were performed.\(^9\)

The timing of surgery also relates to the success of the therapeutic graft. Graft survival in delayed surgeries after early features of scarring was 85.7 %. At the same time keratoplasty in eyes without any clinical response to treatment and emergency keratoplasties showed 40% survival at the end of follow up (p value =0.007). Nobe et al. obtained a graft clarity rate of 17% with emergency surgery (within 24 hours), 57% with intermediate surgery (within 2-6 days) and 31% with a delayed surgery (1 week – 2months).\(^10\) In a study by Foster et al, in eyes that were initially managed with lamellar keratoplasty, or glue and bandage contact lens and a delayed penetrating keratoplasty , a graft clarity rate of 85% was achieved, compared to 17% in eyes treated with an early penetrating graft with perforation.\(^11\)

CONCLUSION

Several surgical interventions have been proposed for treating fungal keratitis, including simple debridement, excisional keratectomy, cover of conjunctival flap and therapeutic penetrating keratoplasty. Our reports support the notion that therapeutic PKP is an effective means of
eradicating the infection and preserving the globe integrity and in many circumstances is inevitable.

Several points of surgical techniques are worth mentioning. The first is the timing of surgical intervention. It should balance the need to minimize the risk of spreading fungal pathogens to a deeper tissue by surgery and the need to restore the globe integrity to minimize the risk of secondary glaucoma. Thus we advise that the therapeutic penetrating keratoplasty be performed after initiation of antifungal drugs for 7-10 days when the cornea is not perforated. This was based on our observation that treatment with systemic and topical antifungal agents for 3-7 days had a significant effect in controlling corneal fungal infection judged by the clearing of the infiltrate margin, drying of the ulcer base, lessening of hypopyon and regression of conjunctival hyperemia. However, if cornea has a large perforation, therapeutic penetrating keratoplasty should be performed sooner. Secondly anterior chamber should be washed and carefully remove the fibrinoid membrane extending onto the iris/lens surface and lyse the anterior synechia as thoroughly as possible. The third point is to perform an iridectomy at the end of the surgery to prevent secondary glaucoma. The fourth point is to avoid removing the crystalline lens even if it appears opaque during therapeutic Penetrating keratoplasty to preserve the iris lens diaphragm so that the spread of fungal pathogens into the vitreous cavity can be prevented.

With the evolution of improved surgical techniques and eye banking the results of therapeutic penetrating keratoplasty in the management of perforated corneal ulcers and other refractory corneal ulcers has improved tremendously. Early surgery has been shown to improve the ultimate outcome of the procedures as well as decrease the hospital stay and morbidity of the patient.

References
Bilateral Acute Angle Closure Glaucoma due to Topiramate

SUMMARY
Systemic anticholinergics, antipsychotics, antihistamines and sulphonamide group drugs are the usual culprits known to induce Angle Closure Glaucoma. Here we present a case of Bilateral Angle Closure Glaucoma induced by Topiramate, a relatively new drug given for conditions like migraine, epilepsy and obesity. Awareness of these drugs and getting the drug history is crucial to the diagnosis as the management is different from Primary Angle Closure Glaucoma. Pilocarpine is contraindicated and YAG PI is ineffective. The most important aspect of management is to identify and stop the offending drug.

CASE REPORT
A 56 yr old male working abroad presented with a 3 day history of pain, watering and redness both eyes. He had consulted his local Ophthalmologist on the first day of symptoms who recorded an IOP 50mmHg both eyes, diagnosed Acute Glaucoma and started Tab Acetazolamide 250mg tds, Brimonidine and Bimatoprost. The patient consulted us as his eyes were still painful. He uses only reading glasses, was a hypertensive and diabetic on medications. He was diagnosed to have Migraine by a Neurologist and started on Tab Topiramate 50mg / day ten days prior to the onset of his eye symptoms.

On examination, his unaided visual acuity was 6/6 OU, IOP 34mmHg OU(by applanation), AC very shallow OU – van Herick <1/4 Corneal thickness (Fig 1), Gonioscopy – closed angles 360° OU (appositional) (Fig 2). Ultrasound Biomicroscopy (UBM) showed a shallow anterior chamber with anterior rotation of iris with ciliary body oedema (Fig 3). Both fundii were normal. Based on the history of Topiramate ingestion, clinical and UBM findings, a diagnosis of Topiramate induced Bilateral Acute Angle Closure Glaucoma was made.

The patient was advised to stop Topiramate and continue Acetazolamide (250mg tds), Brimonidine and Bimatoprost were stopped and replaced with Timolol 0.5% bd. At review 3 days later, IOP was 13mmHg OU (Fig 4) and AC had deepened (Fig 5). Acetazolamide was reduced to bd and Timolol bd continued. At review 7 days later, findings were the same.

The patient was asked to stop the medications after 3 days. A letter was given to the patient to avoid Topiramate and the patient went back to Dubai.

DISCUSSION
Bilateral acute angle closure glaucoma due to Topiramate was first reported in 2001. Topiramate is a sulfamate-substituted monosaccharide given for migraine, epilepsy, depression and obesity. The dose is adult 50-400mg, Paediatric 3-6mg/kg. Tablets are of different strengths - 25, 50, 100 and 200 mg.

Topiramate works by several mechanisms – inhibition of carbonic anhydrase enzyme, blockage of voltage-dependent sodium channels, augmenting the activity of GABA at GABA-A receptor and antagonizing a subtype of the glutamate receptor. Glaucoma and Myopia are believed to be an idiosyncratic reaction to the drug probably mediated by prostaglandins. The mechanism of Glaucoma was shown by UBM to be supraciliary choroidal effusion with ciliary body swelling, anterior rotation and relaxation of zonules with resultant anterior displacement of iris-lens diaphragm. Similar reactions to Sulphonamide, Methazolamide, Oral Acetazolamide and Thiazides have been previously reported.

The diagnosis of this condition is made from a history of recent – less than 1 month – drug ingestion or recent increase in dosage. Thambi et al reported 19 cases, age ranging from 5 to 53 years, symptoms starting at a mean of 10 days after starting Topiramate. Fraunfelder et al in their series of 86 patients had a mean onset of 7 days. Our patient developed his symptoms after 10 days of starting Topiramate 50mg daily. Half of Fraunfelder’s patients were on a dose < 50mg and the other half 50mg and above. In Thambi et al’s series, 3 patients had YAG Laser peripheral Iridotomy with no effect. It is now known that YAG PI is ineffective in these patients as the mechanism is not pupil block. 7 patients in Fraunfelder’s series suffered visual loss highlighting the importance of recognizing this condition.

Patients taking Topiramate tablets should be told to seek immediate medical attention if they experience blurred vision or periorbital pain. Ophthalmologists should suspect this condition in bilateral angle closure cases and in patients < 40 years. They should ask specifically for history of taking Topiramate or similar drugs and whether there

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has been a change in dosage recently. The main treatment is to stop the offending drug, reduction of IOP with systemic medications and drops - Beta blockers preferred, Pilocarpine contraindicated. YAG PI is ineffective. The time for improvement varies from 2 to 14 days. The patient and the treating physician should be given a letter about the condition and other medicines that can give a similar response.

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Dr Thomas Arun Varghese after obtaining his M.S. from Calicut Medical College, worked in UK for 10 years completing FRCS and MRCOPth and then at Little Flower Hospital, Angamaly. Currently he is in charge of Glaucoma and Cataract services at Ahalia Foundation Eye Hospitals at Ernakulam, Paravur and Muvattupuzha.
Spectral Domain Optical Coherence Tomography and Fundus Autofluorescence Findings in Tamoxifen Retinopathy – A Case Report

**PURPOSE:** – To report Spectral domain Optical Coherence Tomography (SD-OCT) and fundus autofluorescence findings in a case of typical tamoxifen retinopathy.

**DESIGN:** – Observational case report.

**METHODS:** – A patient with tamoxifen retinopathy was imaged with SD-OCT and fundus autofluorescence.

**RESULT:** – Spectral domain Optical Coherence Tomography showed numerous hyper reflective spots within the retina, mainly in the inner retinal layers in both the eyes. The external limiting membrane, IS-OS junction and the photoreceptors were not discernable at the fovea in the right eye. In the left eye there was foveal atrophy with total loss of photoreceptors. The auto fluorescent images showed macular hypofluorescence with foveal hyperfluorescence.

**CONCLUSION:** – Spectral Domain OCT demonstrated abnormalities in the outer retinal layers in tamoxifen retinopathy. There was also characteristic alterations in the autofluorescence pattern at the macula in tamoxifen retinopathy.

**CASE REPORT**

A 43 yrs old woman presented with progressive worsening of vision of both eyes for the past six months. She was a diagnosed case of carcinoma of left breast and had undergone modified radical mastectomy. She had also received post operative radiotherapy and six cycles of chemotherapy. The patient was on Tamoxifen 20 mgs/day for the past 4 yrs (cumulative dose of 28 gms). On examination her Snellens visual acuity was 6/18, N8 in both the eyes. Both the eyes had normal anterior segment and intraocular pressure. Dilated fundus examination showed numerous refractile crystals at the macula in both the eyes almost symmetrically.

Spectral domain OCT and fundus autofluorescence were done in both eyes using Spectralis (Heidelberg Engineering, Germany). In the right eye SD-OCT showed numerous hyper reflective deposits at the macula mainly within the inner retinal layers (up to the outer plexiform layer). There was a large hyper reflective spike at the center of fovea projecting into the vitreous. There was also foveal thinning in the right eye (macular thickness was 175 µm). The external

![Color fundus and red free photographs with tamoxifen retinopathy showing refractile deposits at macula.](Image)

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limiting membrane, IS-OS junction and the photoreceptors were not discernable at the fovea in the scan. There was mild macular oedema nasal to the fovea in the right eye.

In the left eye also there were many hyper reflective deposits at the macula in the inner retinal layers. There was a large deposit at the fovea slightly projecting into the vitreous. There was foveal atrophy in the left eye with total loss of photoreceptors (macular thickness was 170 µm in the left eye). There were also few cystoids spaces at the macula nasal to the fovea.

SD – OCT of the right eye showing inner retinal deposits, foveal thinning and the spike like deposit at the fovea. The IS-OS junction could not be seen clearly.

SD – OCT of the left eye showing refractile deposits, photoreceptor atrophy and the large foveal deposit

SD – OCT of the eye showing cystoids spaces

Autofluorescent pictures of both eyes showed generalized reduction of autofluorescence at macula with increased autofluorescence at fovea. The decreased fluorescence at macula may be due to the absorption of fluorescent energy by the deposits and the increased fluorescence at fovea may be because of the unmasking of RPE lipofuschin secondary to foveal thinning. The foveolar spike in the right eye was hypofluorescent probably because the large amount of deposit absorbed so much fluorescent energy that it outweighed the unmasking effect of foveolar thinning.

Autofluorescent images of right and left eyes with tamoxifen retinopathy showing macular hypofluorescence and foveal hyperfluorescence. The foveal spike in the right eye was hypofluorescent

**DISCUSSION**

Tamoxifen is an anti-estrogen drug used most commonly in the management of hormone receptive positive breast cancer. Ocular complications are rare and occur in 0.6% of patients and include cataract, vortex keratopathy, optic neuritis and retinopathy. In the literature, the intake of tamoxifen followed by retinopathy ranges from 6.0 g to 81.0 g for patients with retinopathy. However more recent reports demonstrate maculopathies occurring at much lower cumulative doses (less than 10 gms). Up to 12% of patients taking 20mg/day of tamoxifen develop retinal toxicity. There are also reports of Tamoxifen retinopathy in male patients who were given tamoxifen for inoperable hepato cellular carcinoma. The pathogenesis is thought to be increased accumulation of glutamate which leads to axonal degeneration (observed histopathologically). The crystals seen on fundus examination correspond to the degenerative products. Extensive deposits may result in macular edema and impaired visual acuity. Visual acuity may improve with tamoxifen withdrawal along with resolution of macular edema, but retinal deposits often do not regress.

Gualino et al reported OCT findings in two cases of tamoxifen retinopathy which showed foveal cystoids space, with interruption of the photoreceptor layer without increase...
Kerala Journal of Ophthalmology

256

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in macular thickness which indicated atrophy of the retinal tissue in the fovea. Bourla et al showed macular cystoids spaces associated with fluorescein leakage in one patient and fluorescein leakage without cystoids space in another patient. Both patients showed hyperreflective deposits in the inner retinal layers at the macula and periphery.

Chung et al2 observed intra retinal cysts and focal disruptions of the photoreceptor transition lines on 3D OCT of patients with tamoxifen maculopathy. However the transition line disruption and the exact location and extent of cysts were not visible using TD – OCT in their study. Our case showed numerous hyperreflective deposits at the macula within the inner retinal layers mainly in the nerve fiber layer and plexiform layers which supported the Kupfer et al's suggestion of formation of crystalline deposits due to axonal degeneration. In our case the external limiting membrane, IS-OS junction and the photoreceptor layer were not discernable at the fovea in the SD – OCT. As pointed out by Chung et al these outer retinal details might have been missed by the Time -Domain OCT. In the left eye in addition to many hyper reflective deposits at the macula, there were loss of photoreceptors probably because of the toxic effects of the degenerative deposits. Our case also had few cystoids spaces at the macula as reported by others. The occurrence of large retinal spike like deposit at the fovea projecting into the vitreous seen in our case was not described earlier. This may be due to the higher cumulative dose of tamoxifen. There is a report of foveal hyperfluorescence in tamoxifen retinopathy by Kim et al. Our patient had macular and foveolar hypofluorescence in addition to foveal hyperfluorescence. To the best of our knowledge this is the first report of macular and foveolar hypofluorescence in a case of tamoxifen retinopathy. With the available literature it is recommended that the SD -OCT and autofluorescence screening should be done in patients on tamoxifen with no specific visual complaints or in patients with visual complaints and no specific abnormalities upon routine eye testing. The clear delineation of intraretinal layers by 3D-OCT will surely improve our understanding of the pathogenesis of tamoxifen-retinopathy.

REFERENCES

Viral corneal infections are on a rampage in the clinics. They come in varied disguises and in situations least expected. This entity, in the absence of definitive investigative modalities, needs a sharp intuition and clinical acumen unlike any other ophthalmological case scenario. A few cases with misleading clinical pictures are presented.

Case 1

Herpes simplex viruses (HSV) are ubiquitous and HSV keratitis is the most common cause of corneal blindness in developed nations. It can affect all layers of the cornea primarily or secondarily. Necrotising stromal keratitis and immune stromal keratitis are the two manifestations of primary stromal involvement. A case of the easily misdiagnosed necrotising viral keratitis is presented.

REPORT

A 50 yr old apparently healthy male presented with spontaneous onset pain, redness and photophobia of right eye of ten days duration. He was on commercially available topical antibiotics, antifungals and cycloplegics started from elsewhere but in view of worsening pain and defective vision, was admitted for evaluation.

Slit lamp examination of right eye (Fig 1) showed a central 5×5 mm corneal ulcer with epithelial breakdown and infiltration, epithelial and stromal oedema with descemet's folds surrounding an endothelial plaque 3×3mm size. AC reaction was graded as flare 2+, cells2+, with a white mobile hypopyon of 4mm. IOP was digitally low. Corneal sensation was absent. BCVA was 1mCF. Left eye examination was unremarkable.

The patient was started on fortified antibiotic and cycloplegic eye drops which produced no response. Meanwhile corneal scrapings and cultures turned out negative for bacteria and fungae.

Owing to the absence of response to antibiotics and antifungals and the aggressive clinical picture, necrotizing viral keratitis was suspected. Acyclovir eye ointment was added to the treatment regime. Within days the ulcer improved. The hypopyon resolved, ulcer shrunk and the endothelial plaque gradually disappeared. (Fig2) Antibiotic drops were withdrawn and topical steroids and antiglaucoma medications were subsequently added. Follow up on the third week showed a BCVA of 6/9 OD and a quiet eye.

DISCUSSION

Necrotising stromal keratitis is a rare manifestation of HSV and is due to direct viral invasion of the corneal stroma. The clinical findings of necrosis, ulceration and dense infiltration of the stroma with an overlying epithelial defect resemble those of bacterial and fungal keratitis. Therefore these pathogens must be considered in the differential diagnosis. The combination of replicating virus and severe host inflammatory response leads to destructive intrastromal inflammation that is often refractory to treatment. This may lead to thinning and perforation within a short period of time1 and hence clinical suspicion should be raised early on.

Case 2

An acute presentation of viral keratitis is classically characterised by corneal dendrites, nummular keratitis and geographic ulcers. But this can be representative of a very different aetiology which is frequently misdiagnosed as viral keratitis.

REPORT

A 43 year old lady presented with pain and redness of left eye of three days duration. The eye had sustained trauma with a cow’s tail ten days prior. The patient was already on acyclovir eye ointment, cycloplegics and topical antibiotics from elsewhere but had no relief.

Slit lamp examination of the left eye (Fig 3) showed circumcorneal congestion and a raised dendritic lesion with infiltration up to anterior stroma. The surrounding cornea was
hazy with descemet’s folds. Fine KPs were present. Corneal hyperaesthesia noted. IOP was digitally normal. VA of LE was 2mCF which improved to 6/36 with pin hole. Examination of RE was unremarkable.

Corneal scrapings were negative for bacteriae and fungae. In view of the persistent distressing pain, acanthamoeba keratitis was suspected and neosporin eye ointment was started. The next day she was symptomatically much better. The corneal infiltrate and oedema decreased. Acyclovir ointment was discontinued. Review at two weeks (Fig 4) showed a quiet eye with a dendritic opacity and BCVA of 6/6.

**DISCUSSION**

Severe pain disproportionate to the clinical signs is singularly associated with acanthamoeba keratitis. Other major clinical signs include corneal epithelial irregularity and dendritiform pattern (60%), single or multiple stromal infiltrates (33%), a ring infiltrate (29%) and only 2% manifest with the hallmark finding of radial keratoneuritis. Typically, hyperaesthesia is described in acanthamoeba keratitis but 29% of patients have a decreased corneal sensation. Nummular keratitis, satellite lesions and stromal abscess are described. Thus viral keratitis, bacterial and fungal keratitis come in the differential diagnosis. Minor trauma is associated with dendritic HSV keratitis and Acanthamoeba keratitis but there is a distinct propensity to trauma with soil and water and contact lens wear with the latter. The pseudodendrites and hyperaesthesia in this patient clinched the diagnoses. The dendrite of HSV keratitis is ulcerated with an epithelial defect while the pseudodendrite of acanthamoeba keratitis is elevated with a gelatinous appearance. Healing epithelial defects, herpes zoster dendrites and rarely fungal ulcers also have pseudo dendrites. Herpes zoster dendrites differ from HSV dendrites by being broader, more plaquelike and without central ulceration.

The treatment options available are aromatic diamidines, aminoglycosides, imidazole and triazole antifungals, polymyxins, cationic antiseptics (PHMB) and alkylphosphocholines, administered as a three phased dual drug therapy. Corticosteroids are not advisable. Surgical options include epithelial debridement and penetrating keratoplasty.

**Case 3**

Viral keratitis is notorious for its indolent and relapsing course. A little known albeit treatable sequelae of viral keratitis is reported.

**REPORT**

A 51 year old male presented with recurrent episodes of pain, redness, photophobia of the right eye over two years which temporarily subsides on topical medication, only to recur some time later. He noticed a white spot on the eye four months ago which has been enlarging gradually.

Slit lamp examination of the right eye (Fig 5) showed a paracentral leucoma 5×5 mm with stromal oedema and a surrounding rim of hazy cornea. Lipoid degenerative changes with vascularisation was present. Corneal sensation was decreased. Pupil and fundus were normal. BCVA was 6/18P and N8. Examination of left eye was unremarkable.

He was put on Acyclovir eye ointment and under cover, topical steroids were added. By 40 days’ follow-up, (Fig 6) the leucoma had shrunk and thinned with decrease in oedema and regression of vascularisation.

**DISCUSSION**

Lipid keratopathy may be primary or secondary. The primary form mimics a corneal dystrophy while the secondary form occurs following a viral keratitis or ocular injury. A paracentral or peripheral nummular scar with a vascular leash and lipid deposition in a typical horizontal pattern is seen in 15% of cases 1-2 years after a herpes zoster or simplex keratitis attack. This lipid keratopathy may progress relentlessly to complete corneal opacification. Persistence of the virus in a latent form or persistent reactivation of the virus are mechanisms postulated. Laser photocoagulation, diathermy and needle point cautery of the feeder vessel and penetrating keratoplasty are described treatment modalities. There is a poor consensus on the use of antivirals and steroids.

To conclude, in any microbial keratitis with atypical features and recalcitrant cases, think viral. But all that fits the clinical picture of viral keratitis may not be viral. So keep your senses alert and give an ear to intuition when it comes to treating keratitis.
References


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An 18 yr old male patient presented with gradually progressive defective vision over the past 1 year in both eyes with further diminution of vision in right eye for past 2 weeks. He gives history of seeing flashes of light and occasional floaters. He also gives history of migraine. There is no history of trauma. No history of joint pain, oral or genital ulcers, contact with pets or contact with TB.

On examination : Vn RE -6/12
LE- 6/18
Near vision N8 BE s
BCVA –RE-6/6
LE-6/9
Anterior segment examination --WNL

Fundus examination showed–Multiple yellow deep retinal lesions scattered throughout the posterior pole. In the right eye an yellowish lesion about 1.5 DD was seen at the macula with surrounding localized serous detachment of retina. There was a similar lesion 1 DD temporal to this lesion .In the left eye an yellowish lesion about 1 DD was seen at macula along with smaller yellowish lesions about ½ DD scattered over the posterior pole. There was no evidence of any vitritis in either eye.

IOP -16 mm Hg in both eyes.

Extraocular movements were full in all directions in both eyes.

We made a provisional diagnosis of multifocal Best disease at this stage.

Differential diagnosis of Multifocal choroiditis and Tuberculous granuloma were also considered and hence a complete uveitis workup was done.

Blood inv:

Hb-13, TC-7800 P 54,L-38 E8; ESR-5,Peripheral smear-
mild eosinophilia
Mantoux -10mm
Chest X ray- WNL
ANA –Neg, RA-Neg
Serum ACE-24.7, S.Ca-8.5, Toxo IgG & IgM-Neg,VDRL- NR,
HIV –Neg

FFA was done –multiple hypo & hyperfluorescent lesions which remained same in late pictures also. Hypofluorescent lesions were due to the blockade of fluorescence due to the lipofuscin material .Hyperfluorescent lesions which were not increasing in size was suggestive of staining of the material and atrophy of RPE. These lesions were again suggestive of different stages of Best disease.
OCT was done.

OCT showed thickening of RPE with displacement of photoreceptors and inner layers.
2nd fig shows the typical ‘splitting’ of RPE.

EOG was done which showed only minimally reduced Arden’s ratio in right eye and normal value in left eye.
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Since the condition was not progressive, there was no evidence of any vitritis, the anterior segment was quiet and no evidence of any vasculitis with blood investigations negative. Multifocal choroiditis and TB granuloma were excluded. A diagnosis of multifocal Best disease with pseudohypopyon stage at macula in left eye and vitelliform stage in right eye was made.

His family members were also screened but they showed normal fundus examination and normal visual acuity.

Discussion
Best disease was first reported by German ophthalmologist Freiderich Best who reported the first pedigree of this disorder. Best disease is inherited in an Autosomal dominant pattern. Consequently, the risk of a person transmitting the disease to his progeny is 50%. Sometimes, a new mutation can arise in an affected person. In such cases, all other family members will be normal. Such a person will transmit the disease to the next generation in an autosomal dominant fashion. Also, Best disease may show an intrafamilial variation. Due to variable expression and reduced penetrance, the manifestation of the disease may be so mild that the affected person may be asymptomatic. The age of onset of symptoms also vary within the family. Some members may present early in the childhood whereas others may be asymptomatic till middle age.

Pathology- RPE cells accumulate excessive amount of lipofuscin as evidenced by ultrastructural appearance. This material gets derived from degenerated epithelial cells. The material appears to be more prominent in the macular area. It has been proved that mutations in VMD2 gene which is RPE specific is responsible for the disease. The VMD2 gene codes for the protein bestrophin. Bestrophin acts as a calcium channel and chloride channel regulator.

Best disease has been described as going through 4 phases based on fundus examination. The first stage the previtelliform stage is characterised by a normal fundus appearance. The second stage, the vitelliform stage usually occurs in early childhood and is characterised by a well-circumscribed 0.5-2 disc diameter yellow lesion that looks as an egg yolk and appears to be located in the pigment epithelium. Visual acuity is normal or only slightly reduced. This yellow lesion may eventually breakthrough the RPE into the subretinal space and the yellow material can accumulate in the subretinal space in the macula in the pseudohypopyon stage which is the third stage. The fourth stage is the scrambled-egg appearance where the vitelliform lesions break up and gets scattered.

Complications that can usually occur are choroidal neovascularisation, atrophy, serous detachment of the retina, disciform scarring, macular hole.

FFA reveals blockage of choroidal fluorescence due to the vitelliform lesions. After breakup of these vitelliform lesions there may be depigmentation and staining of atrophic RPE. FFA is not diagnostic of the disease but may add information to correct diagnosis.

EOG is diagnostic for this condition. It usually shows a markedly reduced or nondetectable Arden’s ratio in affected individuals. In a minority of patients, Arden’s ratio may be slightly reduced or normal. Hence a normal EOG cannot exclude Best disease. In multifocal Best disease as in our case EOG may be normal. EOG measures electrical potential across the RPE and hence in Best indicates a diffuse dysfunction of the RPE. Full field ERG is typically normal but multifocal ERG may be abnormal even when visual acuity is preserved.

Till date, there is no causal treatment for this disorder. Management mainly includes genetic counselling and examination of family members. Also follow up of the patients for development of any complications should be done. In case of gross visual loss, low vision aids may be helpful in utilising the remaining vision.

Reference:
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3. Sir Stewart And Duke Elder Disease of Retina
6. DJO 2004 Vol10 No:6 April 24,2004

Present Post: Associate Professor, Regional Institute Of Ophthalmology, Trivandrum
Comparison of deep anterior lamellar keratoplasty and intrastromal corneal ring segment implantation in advanced keratoconus

Yusuf Ozerturk, MD, Esin Sogutlu Sari, MD, Anil Kubaloglu, MD, Arif Koytak, MD, David Pinero, PhD, Sibel Akyol, M


Although penetrating keratoplasty (PKP) and lamellar keratoplasty are still considered the gold standards for the surgical treatment of advanced keratoconus, intrastromal corneal ring segment (ICRS) implantation recently emerged as an alternative surgical treatment in these eyes. The goal of ICRS is to delay and prevent a corneal graft in eyes with keratoconus. The ICRS acts as a passive element that flattens the central cornea by an arc-shortening effect on the corneal lamellar structure.

In this retrospective comparative interventional study, clinical data were retrieved from computerized databases of patients diagnosed with keratoconus who had DALK or ICRS implantation between November 2008 and January 2009 at Kartal Training and Research Hospital.

The keratoconus diagnosis was based on corneal topography. All cases were graded according to the Krumeich system. In the current study, only keratoconic cases with grade 3 (myopia and/or induced astigmatism from 8.00 to 10.00 diopters, mean central keratometry readings more than 53.00 D, absence of scarring, minimum corneal thickness 300 to 400 mm) or grade 4 (refraction not measurable, mean central K readings more than 55.00 D, central corneal scarring, minimum corneal thickness 200 mm) and at least a 24-month follow-up after surgery were included. Exclusion criteria in the ICRS group were a maximum K value greater than 65.00 D, apical opacity and scarring, a history of acute hydrops, vernal keratoconjunctivitis, other ocular disease, and corneal thickness less than 400 mm at the planned site of ICRS implantation (5.0 mm optical zone).

The same surgeon performed all DALK and ICRS implantation procedures.

All DALK procedures were performed under retrobulbar anesthesia using the big-bubble technique. The size of trephination was determined according to the horizontal corneal diameter and the location of the cone.

For ICRS Kera ring segments with 160-degree arc length were used in all cases. An intrastromal tunnel entry incision was created with a 15 kHz Intralase femtosecond laser on the steepest corneal topographic axis. Tunnel depth was set at 70% of the thinnest corneal thickness at the incision site. The inner to outer diameter was set from 4.8 to 5.6 mm.

The ring energy for channel creation and the energy for the entry cut were 1.30 mJ. The ICRS were implanted with a purpose-designed forceps. Uncorrected distance visual acuity (UDVA) and CDVA with a standard Snellen chart, K readings obtained by means of an Orbscan II scanning slit topography system, corneal thickness map, and manifest refraction were measured in all eyes preoperatively and 2 years postoperatively. Decimal Snellen visual acuities were converted to logMAR values for statistical analysis.

The DALK group comprised 37 eyes of 37 patients and the ICRS group, 30 eyes of 30 patients. No intraoperative complications occurred in the ICRS group. In the DALK group, the surgical procedure was converted to PKP in 1 eye because of the presence of a corneal macroperforation; the data for this patient were not included in the statistical analysis. All eyes in both groups completed a 24-month follow-up.

Preoperatively, there were no statistically significant differences in age, CDVA, maximum K, minimum K, SE, and manifest sphere between the DALK group and the ICRS group (p ≥0.09). The increase in UDVA and CDVA from preoperatively to 24 months postoperatively was statistically significant in both groups (p less than .001). Eyes in the DALK group had a greater improvement in UDVA and CDVA than eyes in the ICRS group 24 months after surgery (p less than .001).

In the DALK group, the UDVA and CDVA improved in all eyes (gain 1 to 8 lines) 24 months postoperatively. The UDVA was better than 20/40 in 16 eyes (44.4%), and the CDVA was better than 20/40 in 31 eyes (86.1%). No correlations were found between the postoperative UDVA and CDVA and the preoperative refractive and keratometric values (r between -0.31 and 0.11, P ≥.07).

In the ICRS group, the UDVA improved (gain 1 to 7 lines) in 24 eyes (80.0%), remained unchanged in 3 eyes (10.0%), and decreased (loss 1 to 2 lines) in 3 eyes (10.0%). The UDVA was 20/40 or better in 10 eyes (33.3%). Similarly, the CDVA improved (gain 1 to 6 lines) in 21 eyes (70.0%), remained unchanged in 6 eyes (20.0%), and decreased (loss of 2 lines) in 3 eyes (10.0%). The CDVA was 20/40 or better in 12 eyes (40.0%). No correlations were found between the postoperative UDVA and CDVA and the preoperative refractive and keratometric values (r between -0.19 and 0.11, P ≥.26).

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The improvement in SE, manifest sphere, and manifest cylinder was statistically significant in both groups (p < .001). At 24 months, 20 eyes (55.5%) in the DALK group and 11 eyes (36.6%) in the ICRS group had an SE within ±1.00D of emmetropia. The mean reductions in SE (p = .02) and manifest cylinder (p < .001) were significantly greater in the DALK group than in the ICRS group. The postoperative reduction in the maximum K and minimum K values was statistically significant in both groups (p < .001). The mean reduction in the maximum K and minimum K values was significantly greater in the DALK group than in the ICRS group (p < .001). In the DALK group, an exposed Descemet membrane was achieved in 30 eyes (83.3%) and a layer-by-layer manual stromal dissection was necessary in 6 eyes (16.6%) because of the absence of the big bubble after several attempts. Interface haze was seen in 2 eyes (5.5%). In the ICRS group, no intraoperative complications occurred. The most frequent postoperative complications were limited epithelial defects (27 eyes [90.0%]) on the first postoperative day and sterile white deposits in the corneal channels created for ICRS insertion (19 eyes [63.3%]). The epithelial defects resolved spontaneously during the first postoperative days. Segment extrusion occurred 3 months after surgery in 1 eye (3.3%) with grade 3 keratoconus; the condition was resolved spontaneously during the first postoperative days. Segment extrusion occurred 3 months after surgery in 1 eye (3.3%) with grade 3 keratoconus; the condition was thought to be related to frequent eye rubbing because of the presence of atopy.

The comparison of the results in the DALK group and the ICRS group showed that the corneal flattening and associated refractive correction as well as the improvement in visual acuity were significantly better in the DALK group. The mean change in the absolute value was larger in the DALK group for all parameters evaluated, with statistically significant between-group differences in the UDVA, manifest cylinder, SE, CDVA, maximum K, and minimum K. Therefore, the analysis of the outcomes in the current series study indicate a relative superiority of DALK over ICRS in the management of advanced keratoconus. However, the study also found that ICRS implantation was effective in reducing corneal irregularity without significant complications in eyes with advanced keratoconus. In conclusion, ICRS implantation is a safe and effective procedure for the management of advanced keratoconus, being an alternative treatment option for eyes with this condition but without central corneal scarring. However, the visual impact of ICRS in these ears seems to be less significant than that achieved with DALK.

Five-year clinical study of patients with pseudophakic monovision

Misae Ito, CO, PhD, Kimiya Shimizu, MD, PhD, Yoshihiko Iida, MD, PhD, Rie Amano, MD, MD, Journal of Cataract & Refractive Surgery Volume 38, Issue 8, Pages 1440-1445, August 2012

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Although advances in surgical techniques continually improve modern cataract surgery, the increase in the frequency of refractive surgery has played a part in increasing patient expectations for correcting presbyopia. Despite the introduction of improved refractive multifocal and apodized diffractive multifocal IOLs, changes in pupil diameter can affect visual performance and cause visual discomfort, even with the use of spectacles and contact lenses. The expected accommodative ability is often not attained with accommodating IOLs and the risk for posterior capsule opacification after surgery is high. Therefore, monovision strategies to correct presbyopia remain viable. Monovision correction results in artificial anisometropia, which is an intentionally created difference in the refractive power between the 2 eyes of a patient. The purpose of this study was to assess the 5-year clinical outcomes of pseudophakic monovision in patients with monofocal IOLs.

Patients who had pseudophakic monovision surgery using monofocal IOLs between January 2005 and September 2006 at the Department of Ophthalmology, Kitasato University Hospital, were retrospectively followed. Exclusion criteria were a history of strabismus or amblyopia, clinically significant retinal pathology, glaucoma, optic neuropathy, optic disc anomalies, or other diseases that might affect visual performance. The sighting dominance for distance vision was determined using the hole-in-card test to assess monovision. The nondominant eye was corrected for near vision. Partial coherence interferometry (PCI) was used for preoperative biometry in most patients. A contact A-scan was used for biometry in patients with cataract that was too dense to allow measurements by PCI. The SRK/T formula was used to calculate the IOL power in all cases. Pseudophakic monovision adjustment was determined in all patients after ruling out the following contraindications to pseudophakic monovision: corneal astigmatism more than 1.50 diopters, ocular deviation (strabismus, exophoria more than 10 prism D), or strong ocular dominance. The device used to measure ocular dominance comprised 2 retinometers. A personal computer was used for data storage and analysis. Exclusive visibility of 1 stimulus during binocular rivalry was measured for 60 seconds in each eye. The strength of ocular dominance was evaluated by the difference in the exclusive visibility time between the dominant eye and the nondominant eye and was classified as weak (≤10 seconds) or strong (more than 10 seconds). As a consequence, blur suppression does not function sufficiently in patients with
weak ocular dominance under mesopic vision in which the target appears highly contrasted with the background. Therefore, pseudophakic monovision surgery is not used in patients whose work requires precise operation under low illumination or nighttime driving.

Cataract surgery was performed using topical anesthesia. A monofocal IOL (AQ110NV, Canon-Staar) was implanted using an injector after ultrasonic emulsification through a 2.6 to 2.8 mm temporal corneal incision. The IOL power was selected so that the dominant eye was emmetropic (0 D to +0.25 [SD]) and the nondominant eye was myopic (-2.00D ± 0.50 D). As a rule, an adjustment correction was performed in patients with corneal astigmatism of less than 1.50 D. However, the cataract surgery was performed with a limbal relaxing incision in patients with over 1.00 D of corneal astigmatism who had a strong desire for pseudophakic monovision.

The following postoperative evaluations were performed at 1, 3, and 6 months and 1, 2, 3, 4, and 5 years: logMAR uncorrected distance visual acuity (UDVA), logMAR uncorrected near visual acuity (UNVA), manifest refraction (spherical equivalent [SE]), near stereopsis, ocular deviation, spectacle dependence, and patient satisfaction. Near stereopsis was measured using the Titmus stereo test at 40 cm and ocular deviation was evaluated by the alternate prism cover test while the patient was looking at near without spectacles.

Patients took a survey to evaluate the subjective degree of satisfaction with pseudophakic monovision using a 5-grade scale. Although 96 patients had pseudophakic monovision surgery during the study period, 42 (were lost to follow-up and excluded from the present study. the study evaluated 54 patients (10 men, 44 women) with a mean age of 74.7 years ± 7.9 (SD) (range 51 to 85 years). The binocular UDVA was at least 0.10 logMAR in 53 patients (98.1%), with 41 patients (75.9%) achieving binocular UNVA of Jaeger standard (J2) or better. The mean patient satisfaction score increased significantly from 1 month after surgery to 3 years after surgery (p less than 0.05, Wilcoxon signed-rank test).

The most frequently cited reasons for patient dissatisfaction were asthenopia and spectacle dependence. The mean age of the 5 patients who reported being slightly dissatisfied or dissatisfied was 56.4 ± 3.2 years (range 52 to 60 years), and all reported asthenopia. More than 60% patients had near stereopsis in the normal range. Patients with exophoria of 12 prism or more had a change to intermittent exotropia 2 years after surgery. This method has increasingly become acceptable with each passing year when the degree of satisfaction and the rate of spectacle dependence are considered.

Based on these results, the use of conventional monovision in which the dominant eye is corrected for distance vision and the nondominant eye for near vision can be recommended. However, when strong ocular dominance exists, the resulting anisometropia causes insufficient blur suppression, which results in decreased visual performance; therefore, patients with this condition must be excluded from pseudophakic monovision surgery. Moreover, no vision-threatening complications occurred throughout the follow-up period. Pseudophakic monovision had the long-term stability needed for surgical presbyopia treatment and was an effective approach for correcting presbyopia throughout the 5-year observation period.

Corticosteroids for Bacterial Keratitis
The Steroids for Corneal Ulcers Trial (SCUT)

Muthiah Srinivasan, MD; Jeena Mascarenhas, MD; Revathi Rajaraman, MD; Meenakshi Ravindran, MD; Prajna Lalitha, MD; David V. Glidden, PhD; Kathryn J. Ray, MA; Kevin C. Hong, BA; Catherine E. Oldenburg, MPH;

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The use of topical corticosteroids as adjunctive therapy in the treatment of bacterial corneal ulcers has been debated extensively during the past few decades. The primary objective of the Steroids for Corneal Ulcers Trial (SCUT) is to assess the effect of adjunctive topical corticosteroids on clinical outcomes in patients with bacterial corneal ulcers.

The SCUT is a National Eye Institute–supported randomized, placebo-controlled, double-masked, multicenter clinical trial comparing clinical outcomes in patients with bacterial corneal ulcers receiving topical moxifloxacin, 0.5% (Vigamox); and topical prednisolone sodium phosphate, 1.0% or topical placebo (sodium chloride, 0.9%). Double masking was achieved because the placebo was identical in appearance to the prednisolone sodium phosphate solution. Only the study biostatisticians were not masked. Patients were randomized to receive topical prednisolone sodium phosphate, 1.0%, or placebo after a cornea culture that tested positive for bacteria and after they had received 48 hours of topical moxifloxacin. The prednisolone sodium phosphate and placebo regimens consisted of 1 drop applied topically 4 times per day for 1 week after enrollment, then twice a day for 1 week, and then once a day for 1 week. The moxifloxacin treatment regimen for both arms consisted of 1 drop applied topically every hour while awake for the first 8 hours, then 1 drop applied every 2 hours until reepithelialization, and then 4 times a day until 3
weeks from enrollment. Treating physicians were allowed to change or discontinue the use of any medications, including the antibiotic and study medication, if they thought it was medically necessary. Eligible patients had a culture-positive bacterial ulcer and had received at least 48 hours of topical moxifloxacin before randomization. Major exclusion criteria included corneal perforation or impending perforation, evidence of fungus on potassium hydroxide preparation, Giemsa stain or culture, evidence of acanthamoeba by stain, evidence of herpetic keratitis by history or examination, use of a topical corticosteroid or systemic prednisolone, previous penetrating keratoplasty, and vision less than 6/60 in the fellow eye. Patients were evaluated at baseline, every 3 days ± 1 day until reepithelialization, at 3 weeks, and at 3 months. The primary outcome of the trial was BSCVA at 3 months from enrollment using a tumbling E chart. Secondary outcomes include BSCVA at 3 weeks from enrollment; infiltrate/scar size at 3 weeks and 3 months measured by slitlamp examination; rate of adverse events, including corneal perforation; and time to reepithelialization. Two hundred fifty patients were randomized to receive topical corticosteroid, and 250 received placebo. Four hundred forty-two patients (88.4%) returned for their 3-month follow-up visit within the specified visit window and were included in the analysis. Fifteen patients (3.0%) were excluded from the analysis because they did not return for follow-up in the visit window, and 43 (8.6%) did not return for a 3-month visit. Overall, enrollment characteristics were well balanced between the 2 treatment arms.

For the primary analysis, a multiple linear regression model revealed that corticosteroids offered no significant improvement compared with placebo in 3-month BSCVA, controlling for enrollment BSCVA. Sensitivity analyses did not change this finding. At 3 weeks, corticosteroid-treated patients had 0.024 better logMAR acuity (approximately one-fourth of a line), controlling for enrollment BSCVA (95% CI, −0.082 to 0.044; P = .49). Multivariate regression models showed that corticosteroid use was not associated with a significantly different infiltrate/scar size at 3 weeks (0.05 mm; 95% CI, −0.09 to 0.15; P = .60) or 3 months (0.06 mm; −0.07 to 0.17; P = .40). Median time to reepithelialization was 7.0 days (95% CI, 5.5 to 8.5 days) in the placebo arm and 7.5 days (5.5 to 8.5 days; P = .25) in the corticosteroid arm. Although more patients in the corticosteroid arm had an epithelial defect at 21 days or later compared with placebo (44 [17.6%] vs 27 [10.8%]; P = .04), a survival analysis assessing healing by 3 months showed no difference between the treatment arms (HR, 0.96; 95% CI, 0.81 to 1.16; P = .73). No significant difference was observed in the number of corneal perforations between treatment arms (P = .99). More patients in the placebo arm developed intraocular pressure (IOP) greater than 25 mm Hg but less than 35 mm Hg (P = .04). No IOP elevations above 35 mm Hg were observed in either arm. Forty-two changes or additions in antibiotic were observed in the placebo arm and 34 changes or additions in the corticosteroid arm (P = .38). Seventeen therapeutic penetrating keratoplasties were performed. Subgroup analyses by baseline BSCVA, ulcer location, and infiltrate depth showed a significant effect of corticosteroids (P = .03, P = .04, and P = .04, respectively). In patients with baseline BSCVA of counting fingers or worse, corticosteroid-treated patients had 0.17 better logMAR acuity (approximately 1.7 lines) compared with placebo at 3 months. In ulcers completely covering the central 4-mm pupil, corticosteroid-treated patients had 0.20 better logMAR acuity (approximately 2 lines; 95% CI, −0.37 to −0.04; P = .02) compared with placebo at 3 months. In ulcers with the deepest infiltrates at baseline, corticosteroid-treated patients had 0.15 better logMAR acuity (approximately 1.5 lines; 95% CI, −0.31 to 0.01; P = .07) compared with placebo at 3 months. But the result was not significant. Subgroup analysis by baseline infiltrate/scar size did not show a significant effect of corticosteroids (P = .11).

The SCUT found no significant difference in 3-month BSCVA between patients receiving topical corticosteroid or placebo as adjunctive therapy in the treatment of bacterial corneal ulcers. Before this trial, no conclusive evidence existed regarding the use of corticosteroids for bacterial keratitis. The results of the SCUT demonstrate no obvious benefit in using corticosteroids in the overall study population, also, no apparent serious safety concerns were observed. Noticeably, no apparent increased risk of corneal perforation was incurred with the use of topical corticosteroids. No difference was observed in the number of penetrating keratoplasties in the corticosteroid or placebo arm, suggesting that the use of corticosteroids is not a major concern for the risk of perforation or the need for a therapeutic penetrating keratoplasty. By 3 months, no difference was observed in the rates of healing between patients with ulcers receiving corticosteroid drops vs placebo. An intriguing finding of the study was that prespecified subgroup analyses demonstrated a benefit in 3-month visual acuity using corticosteroids in ulcers with greatest severity at presentation. This is the first large randomized controlled trial to provide evidence regarding the safety and efficacy of the use of corticosteroids in the treatment of bacterial corneal ulcers.

She is currently working as Fellow in Cornea and Anterior Segment, Little Flower Hospital, Angamaly
GLUED IOL

GLUED INTRASCLERAL HAPTIC FIXATION OF A PC IOL

Dr. Amar Agarwal is an international leader in anterior segment surgery who constantly asks and answers the question of how can we improve our surgical techniques to the betterment of our patients. His videos are masterpieces of innovation that have helped to educate an entire generation of ophthalmologists. In addition he is a prolific writer with over 55 books to his credit. Not only he is a superb surgeon with many innovative instrumentations and surgical techniques to his credit, but most remarkably, he possesses the rarest of all personal attributes. Dr. Agarwal, despite all of his accomplishments, is humble and self-effacing, always giving credit to anyone who has in anyway contributed to his training or surgical idea.

One of the most demanding and difficult surgical techniques is to implant an intraocular lens (IOL) in a patient without adequate support for a posterior chamber IOL. An anterior chamber IOL is often a reasonable option but in far too many cases anterior synechiae, glaucoma, or a compromised cornea make this alternative impossible. In addition, anterior chamber IOLs following complex cataract surgery have an increase risk of cystoid macular edema. This left suturing a posterior chamber IOL as the only surgical option prior to Dr. Agarwal’s glued posterior chamber IOL. Suturing a posterior chamber IOL to the iris or through the pars plana to the sclera are both technically challenging procedures and are associated with late complications. An additional option is quite welcome.

The expansion of knowledge in cataract has been truly remarkable as evidenced by the advances and new body of literature. With the glued IOL and intrascleral haptic fixation techniques, secondary IOL implantation has gone to another level. The focus of this textbook has been not so much to express one point of view on the science and handling the secondary IOL fixation, but rather to present a balanced view that is pertinent.

Dr. Agarwal’s invention of the fibrin glued intrascleral haptic fixation of a posterior chamber IOL is a classic example of the prepared mind investigating a new technology. As a premier anterior segment surgeon, he knew the importance of a new technique for implanting a posterior chamber IOL. However, his additional training in vitreoretinal surgery provided him with the tools for microsurgical implantation, through the pars plana under a scleral flap. This approach has been hallmarked of Dr. Agarwal’s career; never accept the conventional wisdom, employ all of your resources and advance patient care.

The Glued IOL book is a comprehensive academic work with exhaustive photographs and an everyday pragmatic guide for general ophthalmologists who intend to manage their posterior capsular breaks with glued IOL technology. Glued IOL is a short form for “Glued intrascleral Haptic Fixation of a posterior Chamber IOL”.

The technique of glued IOL and its modifications along with traditional and cutting –edge surgical interventions are thoroughly explored. The write-ups come from well-known academic institutions and surgeons who are very well-versed with the technique.

The procedure consists of making two partial scleral thickness flaps 180° opposite each other followed by a sclerotomy with a 20 G needle approximately 1-1.5mm away from the limbus; beneath the flaps. After introduction of infusion, the corneal tunnel is fashioned and foldable three-piece IOL implanted. The tip of the leading haptic is exteriorized from one sclerotomy site with the help of a 23 G glued IOL forceps. The trailing haptic is then exteriorized from the second sclerotomy site and the haptics are tucked in the scleral pockets created with the help of a 26 G needle. The flaps are then sealed with the help of tissue glue which provides airtight closure of the wound in the immediate postoperative period.

**Unique features include:**

- Extensive coverage of the history of glued IOL and its evolution
- Technique of glued IOL
- Modifications of glued IOL and its applications
- Nuances of the surgery
- Interactive DVD with recorded videos which highlights the method and give complete description of the technique
- In short, this is a book that every ophthalmologist should have in his/her armamentarium. It is loaded with pearls and practical advice by experts and experienced surgeons.

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The goal was to create a clinically based book and an academic reference that would serve to bring the explosion of new techniques and therapeutic interventions to doctors in trenches who see such patients. This book is non-traditional in several ways. Photos, illustrations and tables are sprinkled liberally throughout the book where appropriate. The book is divided into four sections and 27 chapters by 30 contributors. An interactive DVD showcasing surgeries has also been impregnated into this book.

An attempt has been made to create an informative, useful tool for all the surgeons who are interested in this technique. This should ultimately benefit our patients who place their trust in us for proper management.

Dr. Agarwal’s new book represents the best of his unique and original approach to anterior segment ocular surgery. The book is a comprehensive analysis of the use of fibrin glue to facilitate intrascleral haptic fixation of a posterior chamber IOL. It summarizes all of the best and most useful and practical pearls that he has developed. Dr. Agarwal has brought together and internationally recognized authors and a comprehensive series of videos to demonstrate his signature technique. This book will be widely read by anterior segment surgeons who wish to add to their surgical armamentarium and will be an important contribution to ophthalmology.

Dr. C V Andrews after finishing his MS Ophthalmology and further training from the prestigious BJ Medical College, Ahmedabad went on to do M Phil from BITS, Pilani. He is professor of ophthalmology and medical superintendent at Jubilee Mission Medical College, Thrissur and is currently the president of KSOS

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**OCULAR PROSTHETIC CLINIC**

Following services available in the Department of Orbit, Oculoplasty and Reconstructive Aesthetic Surgery of Giridhar Eye Institute:

1. Custom made prosthesis
2. Stock shell
3. Scleral shell
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5. Pediatric ocular prosthesis

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Ph: 0484-2316791, 4000581,82,83, 2312303, 2324458
Email: girieye@vsnl.com; giridhareye@gmail.com
<table>
<thead>
<tr>
<th>Types</th>
<th>Complaints</th>
<th>Retinal Lesions</th>
<th>Other Findings</th>
<th>Systemic Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Posterior Multifocal Placoid Pigment Epitheliopathy [APMPPE]</td>
<td>Sudden DOV, central or paracentral</td>
<td>Yellow, creamy white lesions at the level of RPE at the macula/ peripheral fundus</td>
<td>AC cells and vitreous cells present. Retinal vasculitis. Optic neuritis</td>
<td>CNS vasculitis. Renal vasculitis. Sarcoid. Thyroiditis</td>
</tr>
<tr>
<td>Multiples evanescent white dot syndrome – MEWDS</td>
<td>Acute loss of vision. Photopsia. Enlarged blind spot. Self limiting course.</td>
<td>Multiple small indistinct white spots all over the posterior fundus. Each dot is composed of aggregates of smaller dots at the level of RPE.</td>
<td>Varying AC / vitreous cells</td>
<td>–</td>
</tr>
<tr>
<td>Serpiginous choroiditis</td>
<td>Blurred vision. Photopsia. Metamorphopsia. Central / Paracentral Scotoma</td>
<td>Sharply demarcated grey, green/ cream colored jig- jaw puzzle shaped lesions of varying size involving RPE and choriocapillaries.</td>
<td>AC reaction and vitritis</td>
<td>No systemic association</td>
</tr>
<tr>
<td>Bird shot retinochoroidopathy</td>
<td>Varying degree of gradual painless visual loss. Floaters. Colourvision disturbances. Nyctalopia</td>
<td>Multiple bilateral round and oval depigmented yellow white spots varying in size with relatively indistinct borders in the posterior pole and post equatorial fundus. Blond appearance of fundus in case where geographic depigmentation occurs.</td>
<td>Varying AC reaction</td>
<td>–</td>
</tr>
<tr>
<td>Mutifocal chorioiditis and panuveitis syndrome MCP</td>
<td>Decreased vision. Floaters, photopsia. Enlarged blind spot</td>
<td>Multiple round to oval yellow gray lesions at the level of RPE scattered in the posterior pole and mid periphery. Later evolve into chorioretinal scars with a punched out appearance.</td>
<td>Vitritis more than AC reaction</td>
<td>–</td>
</tr>
<tr>
<td>Punctate inner choroidopathy PIC</td>
<td>Loss of central visual acuity Photopsia</td>
<td>Lesions similar to MCP, concentrated in the posterior pole.</td>
<td>No uveitis – Hallmark</td>
<td>–</td>
</tr>
<tr>
<td>FFA</td>
<td>ICG</td>
<td>COMPLICATIONS</td>
<td>OTHER TESTS</td>
<td>TREATMENT</td>
</tr>
<tr>
<td>-----</td>
<td>-----</td>
<td>---------------</td>
<td>-------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Early phase – Hypo (blockade due to swollen RPE or choroidal non filling). Late Phase- Hyper (staining)</td>
<td>Choroidal hypofluorescence in early and late stages. Perfusion abnormalities in choriocapillaries persists to late stage</td>
<td>Vasculitis</td>
<td>ERG, EOG Abnormalities</td>
<td>Self limiting disease. Steroids in case of vasculitis</td>
</tr>
<tr>
<td>Early punctate hyperfluorescence. Late deep staining in a wreath like cluster .</td>
<td>Early phase – normal. Late phase (&gt; 10 mts) lesions appear as multiple hypofluorescent spots more in the posterior pole radiating to periphery.</td>
<td>Optic nerve head swelling. Vascular sheathing. Rarely CNVM</td>
<td>ERG -a wave ERP abnormalities EOG- abnormal. VEP - Abnormal if optic nerve is involved</td>
<td>Not required usually.</td>
</tr>
<tr>
<td>Early hypofluorescence and late hyperfluorescence. Brush fire staining</td>
<td>More extensive hypo in early and late stage. Diffuse atrophy of choriocapillaries</td>
<td>ERG / EOG – normal except in extensive disease.</td>
<td>Triple therapy. Steroids/Cyclosporin Azathioprin</td>
<td>Poor</td>
</tr>
<tr>
<td>Lesions may remain silent in angiogram due to deep location or early evolution. If RPE choriocapillaries are involved, early hypo and late hyperfluorescence. Vascular/ Disc leakage .</td>
<td>Well demarcated hypofluorescent choroidal spots with vasotropic orientation bordered by large to medium choroidal vessels .</td>
<td>ERG – Disproportionate decrease in b wave with relative sparing of a wave . VEP- decreased amplitude and delayed latency suggesting intrinsic ONH dysfunction</td>
<td>Steroids / Immunosupression</td>
<td>Chronic</td>
</tr>
<tr>
<td>Active lesions may be non fluorescent in early phase with gradual staining and late leakage</td>
<td>Hypofluorescent spots</td>
<td>Exudative RD during development of spots. Serous RD CME CNVM Retinal vasculitis and neovascularisati on</td>
<td>ERG- Both a and b waves affected . Visual fields- Enlargement of blind spot.</td>
<td>Immunosupressents/ Steroids</td>
</tr>
<tr>
<td>Hyperfluorescence in A-V phase with late leakage .</td>
<td>Hypofluorescent spots</td>
<td>CNVM</td>
<td>Visual field – Enlarged blind spot ? Steroids</td>
<td>May develop subretinal fibrosis</td>
</tr>
</tbody>
</table>
Name this great Nobel Laureate?  
What did he invent in ophthalmology?

Send your answers to gopalspillai@gmail.com
The First Correct answer gets the prize

Last time's winner is Dr Abhishek from SMCSI Medical College, Trivandrum
The answer is "Best's disease Diagnostic tests are ERG and EOG"
General Instructions To Authors

The Kerala Journal of Ophthalmology (KJO) is a quarterly, peer reviewed one, devoted to dissemination of the latest in ophthalmology to the General Ophthalmologists as well as to specialists in the various subspecialties of this discipline. It invites submission of original work dealing with clinical and laboratory materials.

Authors submitting materials to this journal are requested to adhere STRICTLY to the norms laid down below. The matter must be typed on one side of the paper. A margin of 1” must be left all around and the material must be double spaced. A page should contain not more than 25 lines. Two copies of the text in paper and one copy in a CD must be submitted to the Editor and the corresponding author is advised to keep another copy with him. The corresponding author must give it in writing in his covering letter that the same matter will not be submitted elsewhere if accepted. He must also enclose the copyright transfer of his work to this journal. The papers sent will be subjected to peer review. The accepted manuscripts become the permanent property of this journal. The author is informed that, if his work is returned to him for correction / clarification after peer review, he should effect the same and send the manuscript back to the Editor within one month. Each manuscript component mentioned here under must begin with a new page and the pages are to be numbered at the right tip corner starting from the Title page.

1. TITLE: The title of the work must be brief and precise. It should not exceed two lines and 40 characters (including comma, period) Author(s) full name (s) must be given along with his (their) degree and the affiliations. Corresponding author’s name, correct address (including e-mail and Fax, if available) and phone number must be mentioned at the bottom left hand corner of the first page.

2. ABSTRACT: The abstract is to be given in the beginning itself. It should not exceed 200 words. It must contain the aim, methodology, results and conclusion. For case report, summary / conclusion alone is to be given.

KEY WORDS: (maximum five) in capitals are to be included at the end of Abstract.

3. INTRODUCTION: Describe the aim of the study, along with the hypotheses that were tested. Only necessary references are to be given.

4. METHOD: Give in detail the materials used and the methods employed. Describe the type of study. Pharmacological names only must be mentioned for the drugs used and, if proprietary name is used, then the manufacturers name must be given in parentheses. Except for standard, well-accepted abbreviations (including SI Units), all others must be introduced in parentheses when the full term is used for the first time in the article.

5. RESULTS: Give only the results obtained by the study under discussion. State the statistics in the correct scientific form (P value, mean etc). Results based on assumptions must not be given. Indicate in the text the place where the tables have to be inserted.

6. DISCUSSION: The discussion should be to the point and relevant to the subject under discussion. This section can be combined with the previous one if the author desires. Avoid speculations. Use only standard abbreviations or the abbreviations already introduced.

7. ACKNOWLEDGEMENT: This is to be made only to those who were directly and scientifically involved with the preparation of the paper. Permitting authorities, technicians, photographers who assisted in the work need not be mentioned.

8. REFERENCES: The references should be given in numerical order in which they first appear in text and not in alphabetical order (Citation Order System). It should be numbered consecutively in the text. The references will not be checked by the Editor or by the Peer reviewer and hence the author is solely responsible for its completeness and the accuracy. Period should not be employed anywhere in the references. Personal communications, unpublished data and poster references, if mentioned, should be in the text itself and the source mentioned in parentheses. References should be in the following form:

Journal reference: Author(s) full title, Journal name (as abbreviated in Index Medicus), volume number, pages and year. If there are more than three authors, then mention the first three authors and then ‘et al’.

Book reference: Author(s) (& Editor, if any), title of book (and chapter), publisher, place of publication, page number (s) of the cited portion and year.

9. THE LEGEND: The legend for the illustrations (and tables, if necessary) must be given in a separate sheet of paper and should be typed double-spaced.
Illustrations: The photos and figures should be prepared in glossy prints with good contrast and of the size 6" x 4". Only salient details should be included. On the back of the illustration, the figure number in text, title of the paper, the first author’s name and the top side (marked with an arrow) must be specified. Except for arrows, no text is to be on the photos. It is the duty of the author(s) to get the patient’s written permission when the subject is identifiable in the photo. Submit two sets of illustrations. Illustrations from other Journals and books are usually not accepted. If used, it rests with the author(s) to get the copy right permission from the original author / publisher and this permission letter must be sent to the Editor at the time of submitting the manuscript. For Histological figures the stain and magnification used should be noted e.g.: H & E Stain x 70.

10. TABLE: It should be in double space. Each table must have an Arabic numeral (except for single table) and a title both in a single line. Each column in the table must have a short heading. If a table is large, then it must be continued in a second page, which also must have the table number and the title. Avoid vertical lines in the tables. Two sets must be submitted.

11. All manuscripts are subjected to editorial board review.

12. Other Categories of Manuscript

a) Original Articles should generally not exceed 3,000 words or 12 double – spaced pages.

b) Review Articles: can be on topics of relevance to clinical practice, research methodology, community ophthalmology or investigative work, of relevance to visual science. These articles should include up to date review of existing literature, and summarize the current status / preferred practice for that particular topic.

Brief reports are short communication of new instruments, new laboratory techniques or surgical techniques as well as interesting case reports with unique findings. These should not exceed 1000 words with a maximum of 2 illustrations. They should follow the format – introduction, case, and discussion. No more than 8 references should be cited. Each brief report must begin with a 75-100 word summary that highlights the significance of the articles.