"A ONE YEAR PROSPECTIVE STUDY TO EVALUATE THE EFFICACY OF AMNIOTIC MEMBRANE TRANSPLANT AS AN ADJUNCT TO TRABECULECTOMY IN PATIENTS ADMITTED IN KLES DR. PRABHAKAR KORE HOSPITAL & MEDICAL RESEARCH CENTRE, BELAGAVI"

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ACCEPTANCE LETTER

The softcopy of thesis entitled: **"A ONE YEAR HOSPITAL BASED LONGITUDINAL STUDY TO EVALUATE THE SAFETY AND EFFICACY OF USING AMNIOTIC MEMBRANE AS AN ADJUVANT TO TRABECULECTOMY."** has been submitted for Anti-Plagiarism check through Turnitin software. The scan has been carried out and the scanned output reveals a match percentage of **05%** which is within the acceptable limits of **10%** as per the guidelines given by UGC.

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LIST OF ABBREVATIONS

IOP	:	Intraocular pressure
5-FU	:	5-Fluorouracil
MMC	:	Mitomycin- C
TGF β	:	Transforming growth factor β
AC	:	Anterior chamber
PC	:	Posterior Chamber
AMT	:	Amniotic membrane transplantation
POAG	:	Primary open angle glaucoma
PACG	:	Primary angle closure glaucoma
TM	:	Trabecular meshwork
BCVA	:	Best corrected visual acuity
PCIOL	:	Posterior Chamber Intraocular Lens
TASS	:	Toxic Anterior Segment Syndrome
BSS	:	Balanced Salt Solution

ABSTRACT

Purpose:

To evaluate the safety and efficacy of amniotic membrane transplantation as an adjunct to trabeculectomy in glaucoma patients at a Tertiary Care Hospital located in Southern India.

Methods:

This was a prospective, longitudinal, interventional, hospital based study conducted in a Tertiary Care Hospital located in Southern India. Thirty one patients who visited the out-patient department and diagnosed with primary glaucoma underwent trabeculectomy with amniotic membrane transplantation and were assessed postoperatively upto 3 months.

Results:

Out of the thirty one patients, one patient was lost for follow up due to the COVID-19 pandemic and thus excluded from the study. 25 patients (83.33%) had complete success while the remaining 5 patients (16.67%) had qualified success. All 30 patients (100%) had total success with a significant reduction in IOP All blebs were diffuse with moderate or low height with majority patients having a central area of 25% and paiphual area of 25%, raised and mildly vascular. Complications observed were 2 patients (6.67%) had incomplete peripheral iridectomy, 1 patient (3.33%) had sub conjunctival haemorrhage under the bleb and 1 (3.33%) had hyphaema on post-operative day 1, both of which resolved by the 4-week follow up, posterior capsule rent occurred intraoperatively in 1 patient (3.33%) which was managed by placing PCIOL in sulcus, and 1 patient (3.33%) had toxic anterior segment syndrome which

was treated with topical and oral steroids. These complications did not show any significant difference in postoperative IOP or bleb morphology.

Conclusion:

Trabeculectomy with amniotic membrane transplantation is both safe and efficacious. The reduction in intraocular pressure was clinically significant with a p-value of <0.001 and a good bleb morphology of diffuse and mild vascularity by the end of 3 months with all patients having total success.

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INTRODUCTION

Glaucoma is an irreversible group of diseases progressively modifying the integrity of the optic nerve leading to typical patterns of visual deformities. It is an acquired optic atrophy associated with continued damage to ganglion cells of retina and their axons, manifesting as distinctive visual field changes.(1)

According to World Health Organization, glaucoma is the second highest reason of blindness in the world following cataract.(2) The estimated global burden of glaucoma is around 79.6 million as of the year 2020, with almost half of them being Asian. The global prevalence of glaucoma is estimated at 3.54%.(3)

The word glaucoma is derived in 400 BC from a Greek word 'glaucosis' meaning clouding of the affected eye termed for a group of disorders resulting from persistent raised ocular pressure either due to a mature cataract or due to edema of cornea.(4)

There are two schools of thought contributing to glaucomatous damage. First being the ***mechanical hypothesis*** which states that ocular hypertension leads to structural damage to the retinal nerve fibre layer and eventually impedes orthograde & retrograde axoplasmic flow, thus destroying the ganglion cell body. Second is the ***mechano-vascular hypothesis*** which says that compression of the microvasculature of the optic nerve head results in ischemia and subsequent destruction of the nerve fibre layer.(5) Among various mechanisms, elevated intraocular pressure is the most common contributory risk factor for glaucomatous optic nerve damage.(6)

Although other factors may be contributory, the currently available treatments are aimed at lowering the intraocular pressure (IOP) to a targeted intraocular pressure. The goal must be to reduce the IOP to a level that is 'safe' for that individual eye.[4]

This "target IOP" can be determined by the optic nerve changes and/or visual field loss, baseline IOP at which damage occurred, and patient's age. This may be achieved by antiglaucoma medication, laser or filtering surgical management, and cyclodestructive procedures.[6]

The first-line therapy is ocular hypotensive therapy which is started after considering various factors such as affordability, risk/benefit ratio of lifelong medications, quality of life, side effect of medications, compliance and follow-up by patients.[5] This is then followed by trabeculectomy or other surgical approaches as a second-line mode of management in case of extremely high uncontrolled IOP, poor compliance to topical medications or inability to tolerate topical medical treatment.[7]

Trabeculectomy, the gold standard surgical intervention creates a new outflow pathway for drainage of aqueous humor from anterior chamber (AC) into subconjunctival space. The aqueous then enters subconjunctival vessels and lymphatics or in case of a bleb with thin walls, passes through conjunctiva and into the tear film.[8] The aim of this is to stabilize the existing clinical picture by retarding the worsening of disease.

However, for a successful outcome of surgery, the physiological wound healing process needs to be interrupted. The proven cause for failure of surgery is proliferation of fibroblasts which results in episcleral and subconjunctival fibrosis. To

sustain the patency of this newly fashioned pathway, the advent of antifibrotic agents came about in the early 1980s such as corticosteroids , beta irradiation, 5-fluorouracil (5-FU) and Mitomycin- C (MMC) which have seemingly improved the surgical outcomes.[8]

Their well-known complications such as avascular blebs, oozing transconjunctivally, ultimately resulting in loss of endothelial cells, cataract, hyphaema, flat AC & hypotony, suprachoroidal haemorrhage, hypotonus maculopathy, malignant glaucoma, blebitis, intraocular infections and endophthalmitis have consequently steered the search towards a safer and more physiological bleb modifying alternative while simultaneously reducing the complication rate. This lead to the consideration of amniotic membrane transplantation in trabeculectomy.[9]

Amniotic membrane transplantation (AMT) stimulates epithelialization by acting as a scaffold and an appropriate basement membrane for epithelial cells to grow on and also impedes fibrosis by downregulating transforming growth factor β (TGF β) signaling and myofibroblast differentiation.[10]

Human amniotic membrane has a well established anti-angiogenic, anti-inflammatory, and antifibrotic properties including good incorporation with surrounding tissues, low healing response, poor immunogenicity, and yet a high hydraulic conductivity. These characteristics might be of benefit in filtering bleb construction.[11]Then again, like any biological substance transplantation, amniotic membrane also carries the risk of transmitting infections which is minimised by serological testing of donor placenta for communicable diseases like HIV, syphilis and hepatitis.[12]

According to various experimental and clinical studies, transplantation of amniotic membrane in trabeculectomy has shown encouraging results in IOP control. However, its usage and efficacy as a usual practice to prevent subconjunctival scarring remains unclear.[12]

Therefore, the purpose of our prospective baseline study is to evaluate the effectiveness of Amniotic membrane transplantation in trabeculectomy for the treatment of glaucoma.

AIMS AND OBJECTIVES

AIM

To evaluate the safety and efficacy of amniotic membrane transplantation as an adjunct to trabeculectomy in glaucoma patients at a Tertiary Care Hospital located in Southern India.

OBJECTIVES

Primary Objective

To evaluate the efficacy of amniotic membrane transplantation in trabeculectomy.

Secondary Objective

To evaluate the outcomes and complications of amniotic membrane transplantation in trabeculectomy.

REVIEW OF LITERATURE

HISTORY OF GLAUCOMA

In the **4th century** BC of the Hippocratic era, the term "glaykoseis" was used to describe glaucoma as blindness affecting the elderly and associated with a glassy pupil – "if the pupil becomes sea-coloured, sight is destroyed and blindness of the other eye often follows."

The first indication of a disease associated with a rise in intraocular pressure, now identified as glaucoma seems to have appeared in Arabian writings in the "Book of Hippocratic treatment" in the **10th century**.

In the **15th century**, a European ophthalmologist named Dr. Richard Bannister clearly differentiated between "gutta obscura" (cataract) and "gutta serena" (glaucoma) and gave a tetrad consisting of –"tension of the eye; long duration of disease; absence of perception of light and the presence of a fixed pupil".

In the **16th century**, Charles Saint-Yves defined glaucoma as an acute inflammatory condition of the eye wherein the vision became hazy, pupil turned sea-coloured, severe pain and visual field showed defects where objects were seen partially.

In the **17th century**, Antione-Pierre Demours noted and described for the first time the manifestation of rainbow colours around lights in this disease. The principal concept of elevated intraocular pressure later became well established by William Mackenzie in 1835 who attributed the elevated pressure to both acute and chronic (congestive) glaucoma.

In the **19th century**, Heinrich Muller noted optic disc cupping in association with raised intraocular pressure. Von Graefe then went on to categorize glaucoma into- ***acute, chronic and secondary*** which was then modified by Donders in 1862 with his unifying concept, wherein he recognized a final condition with an incapacitating rise in tension that occurred without any inflammatory signs as **'*simple glaucoma'***.[13] In the **20th century**, Dr. Drance defined glaucoma as an optic neuropathy caused by numerous factors.

Now, the established ***definition of glaucoma*** is - disturbance in structural and functional integrity of optic nerve that can usually be arrested or diminished by adequate lowering of IOP.[4]

EPIDEMIOLOGY OF GLAUCOMA

The burden of glaucoma in the world is estimated to be around 70 million; out of which, 7 million are blind. With respect to India, glaucoma prevalence is 0.5 to 1.5% amongst all ages and 2.5 to 4.5% in the elderly across various regions.[14]

Approximately, 57.5 million people worldwide are affected by primary open angle glaucoma (POAG) with a prevalence of 2.2%.[15] In Europe, 7.8 million people having POAG and a prevalence is 2.51%[2,3,16]. Of all the glaucomas, POAG is the most prevalent one in the United Kingdom impacting 2% of the population over 40 years of age and 10% of the population over 75 years, specifically Afro-Caribbean population.

Primary angle closure glaucoma (PACG) only involves 0.17% of the population below the age of 40 years, predominantly in Eastern Asia.[17] A study has

implied that disparities in socioeconomic conditions have an impact on the available glaucoma facilities.[15,16,18]

ANATOMICAL AND PHYSIOLOGICAL ASPECTS

AQUEOUS HUMOR

Aqueous humor is formed by the ciliary processes at a rate of 2-3 μl/min and flows from the posterior chamber to AC via pupil, finally exiting at the angle of AC. This rate decreases by 2% every decade and by 45% during sleep.

Aqueous is produced by three mechanisms- ultrafiltration, active transport and diffusion.

1. Ultrafiltration

Blood flows through the fenestrated capillaries of the ciliary processes into interstitial spaces between capillaries and ciliary epithelium along a pressure gradient.[4] The hydrostatic pressure difference between IOP & capillary pressure favours movement of aqueous into the eye, while the high concentration of colloids resists it.

2. Active Transport

It is energy dependent. It actively pumps over 70% of sodium reaching the aqueous. This sodium gradient thus creates an osmotic gradient drawing majority of the water into the aqueous.

3. Diffusion

As aqueous moves from posterior chamber (PC), through the trabecular meshwork (TM) into Schlemm's canal, there is sufficient diffusional exchange of lipid soluble molecules such as oxygen, glucose, certain electrolytes and amino acids to the adjacent tissues while eliminating lactate, pyruvate and CO_2.[4]

Functions of aqueous humor

- Provides nutrition and oxygen to cornea, iris and lens and removes products of metabolism generated by them.
- Offers an optically sharp medium for a good visual acuity.
- Maintains intraocular pressure of the eye, thus preserving its structural intergrity.
- In response to inflammation and infection, facilitates cellular and immune defense mechanisms.
- Scavenges free radicals in response to ultraviolet radiations with the help of high ascorbate levels.[4]

Aqueous humor outflow

Occurs via two mechanisms-

- Major pathway- pressure-dependent Trabecular outflow.
- Minor pathway- pressure-independent uveoscleral outflow.

Trabecular outflow or conventional pathway contributes to 83-96% of aqueous drainage through the TM flows into Schlemm's canal & finally into venous system through a plexus of collector channels.

Uveoscleral outflow or unconventional pathway contributes to 5-15% of the drainage.

It occurs through the iris root and ciliary body to reach the supraciliary and suprachoroidal spaces.(1)

Anatomy of canalicular/trabecular pathway

Schwalbe's line

An irregular elevation composed of collagen and elastin that runs around the globe which is considered as Descemet membrane's termination denoting the transition from TM to endothelium of cornea. Secretory cells present in this zone enable aqueous outflow.

Scleral spur

A fibrous ring made of type I & III collagen and elastic tissue arranged in a circumferential manner. It is affixed to TM anteriorly and longitudinal portion of ciliary muscle and sclera posteriorly such that on contraction of ciliary muscle, it retracts the scleral spur. This leads to enlarged intertrabecular spaces, thus increasing the size of lumen of Schlemm's canal, reducing its tendency to collapse.(4)

Trabecular meshwork

It is a spongy sieve like tissue, triangular in shape on meridional section, Schwalbe's line forming its apex and scleral spur forming its base. It consists of 2 parts: nonfiltering and filtering.

I. ***Non filtering part***: It lies just posterior to Schwalbe's line, forming the frontal portion of TM and consists of three to five trabecular beams covered by small trabecular cells that form elongated bands or rows.

II. ***Filtering part***: It is split into 3 parts

a. Uveal meshwork- Inner portion, consists of strands covered by endothelial cells originating from iris and stroma of ciliary body. The intertrabecular spaces are large and offer less resistance to the flow of aqueous.

b. Corneoscleral meshwork- Thickest part of meshwork extending from scleral spur to anterior wall of scleral sulcus. It consists of series of 8-14 flattened perforated parallel sheets of lamellae with a thickness of 5-12µ. The intertrabecular spaces are smaller than those of uveal meshwork, conferring relatively greater resistance to passage of aqueous.

c. Juxtacanalicular space and cells- This 2-20µ thick cribriform network forms the outer part of meshwork. It connects corneal meshwork with Schlemm's canal inner wall. The pore size in fixed tissue was found to be 0.5-1.5µ offering major proportion of resistance to aqueous outflow.

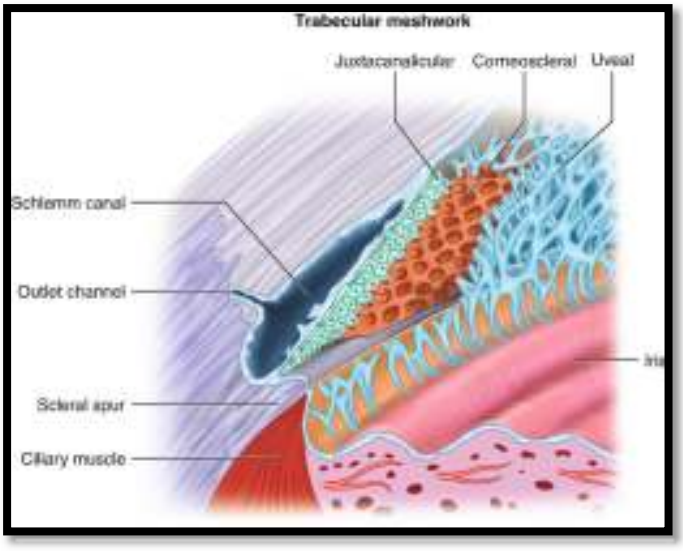


Fig 1- Three layers of the filtering part of trabecular meshwork (in cross section) Juxtacanalicular, corneoscleral and uveal.

Schlemm's canal

Canal of Schlemm is a vascular sinus lined by endothelium consists of a hollow area that is interconnected and surrounds the globe. The canal's diameter is IOP dependent.

Collector channels, aqueous veins and episcleral veins

A series of collector channels pour aqueous into aqueous vessels, the aqueous veins of Ascher originating from outer wall of Schlemm's canal and terminating in subconjunctival/episcleral venous plexus.

Through anterior ciliary and superior ophthalmic veins, these episcleral veins then pour into the cavernous sinus. Through palpebral & angular veins, the conjunctival veins pour into superior ophthalmic or facial veins.[4]

Aqueous outflow physiology

As a passive filter- This describes the aqueous flow as a non-ATP-dependent movement of fluid exiting the eye via traditional Schlemm's canal. This can be explained by aqueous driven through the juxtacanalicular meshwork which provides resistance passively to the movement of aqueous.

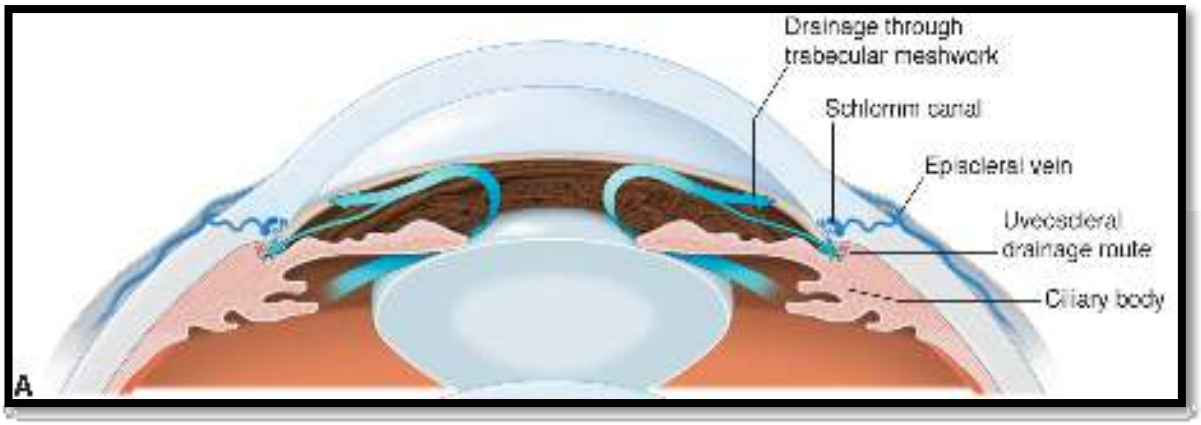
As a dynamic mechanical pump- This system is that of a biochemical pump mechanism. In response to transient pressure (IOP) increases caused by ocular pulse, blinking and eye movements, the contractile component of TM and valves of Schlemm's canal is subject to stretch. Energy stored in distension is released as pressure decay occurs while the tissues recoil to the original configuration. This energy thus enables energy-dependent pulsatile fluid movement.

Anatomy of Uveoscleral outflow pathway

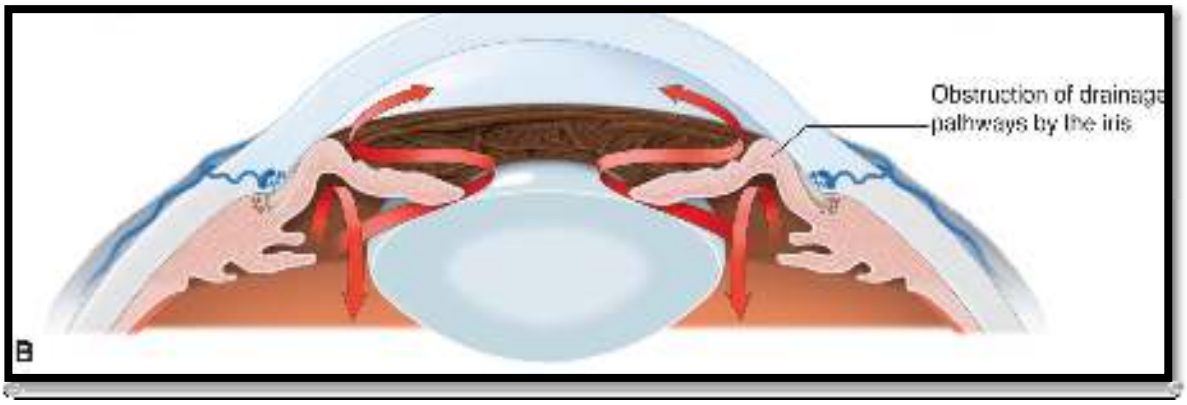
A lesser amount of aqueous via the uveal meshwork flows into ciliary muscle, ciliary body and root of iris and drains via the suprachoroidal space & supraciliary space. Aqueous exits eye via the area around vessels and nerves piercing the globe and through sclera.

Only a small amount of fluid drains via this pathway due to various reasons:

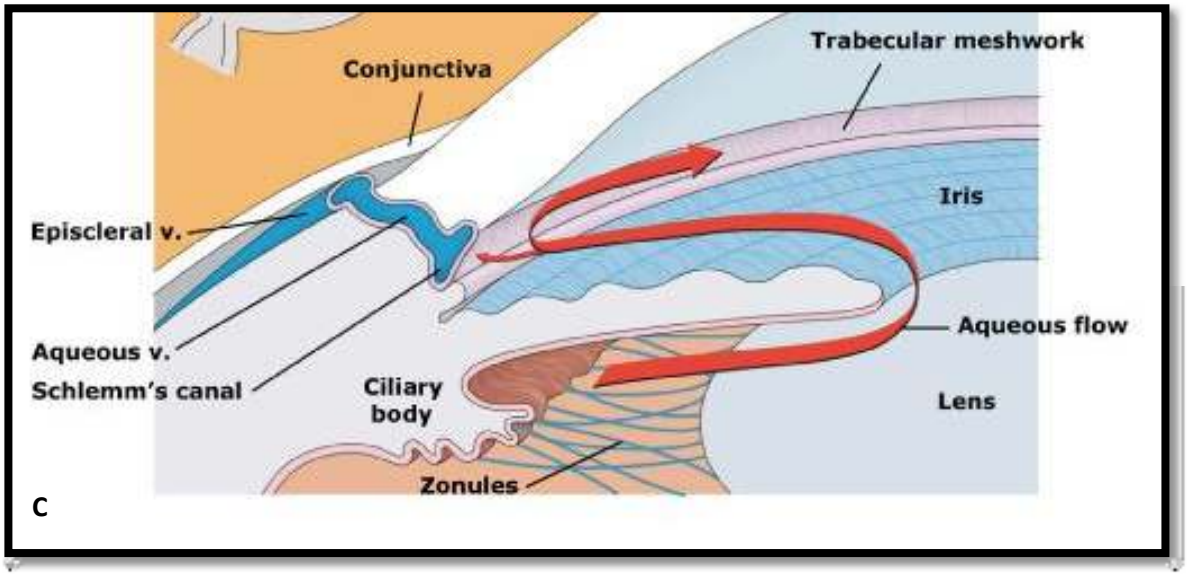
- Thick walls of iris capillaries that prevent the motion of ions & water.
- Pressure gradient created by the difference in pressures- which is greater in uveal capillaries as compared to intraocular pressure.
- The variation in oncotic pressure between aqueous in uveal tract and plasma, create a minimal propelling force for aqueous to pass through the walls of capillaries.



A. Normal outflow via trabecular meshwork (large arrow) & via uveoscleral pathway (small arrow)



B. In ACG, the abnormally positioned iris blocks aqueous outflow through the angle of anterior chamber



C. In POAG, aqueous outflow by these pathways is diminished

Fig 2- Aqueous outflow

ANATOMY OF LIMBUS AND ANTERIOR CHAMBER ANGLE

SURGICAL LIMBUS

It is a transitional zone with a width of 2mm, present circumcorneally. It is bounded by cornea on one side while sclera is on the other side. Surgical limbus is divided into two zones by three borders.

Borders of surgical limbus-

1. ***Anterior limbal border***- Prominent ridge marked by the insertion of conjunctiva and tenon's capsule into the cornea. It overlies the termination of Bowman's membrane. It forms the anterior border of the surgical limbus.
2. ***Mid-limbal line***- It overlies the Schwalbe's line (termination of Descemet's membrane).
3. ***Posterior limbal border***- It overlies the scleral spur. It is 1mm posterior to mid-limbal line and can be viewed using sclerotic scatter illumination during slit lamp examination.

Zones of surgical limbus-

1. ***Blue limbal zone***- On dissection of limbus free of conjunctiva and Tenon's capsule, a bluish semitransparent zone is exposed behind the anterior limbal border and anterior to the mid-limbal line . The extent of the zone varies in all quadrants: 1mm superiorly, 0.8mm inferiorly, 0.4mm in nasal and temporal quadrants.
2. ***White limbal zone***- A 1mm wide zone between mid-limbal line and posterior limbal border. It overlies the TM and the breadth of this zone is even in all quadrants.

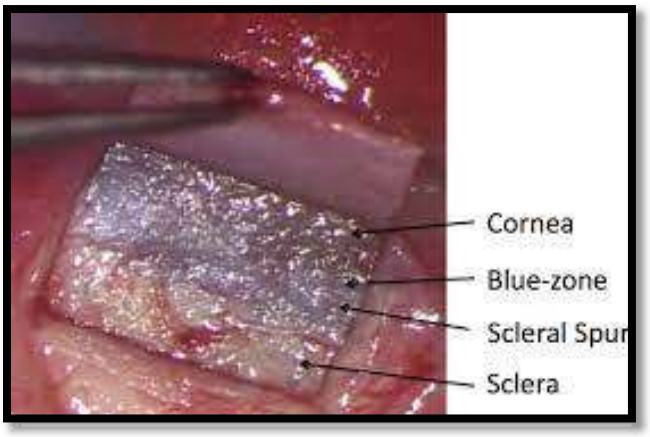


Fig 3- Zones of surgical limbus

Applied anatomy

The posterior border of the blue zone is analogous to the location of trabecular meshwork internally. Thus, incisions placed anterior to the blue zone would enter away from the trabecular meshwork.

ANGLE OF ANTERIOR CHAMBER

Structures forming angle recess

Angle of anterior chamber visualized by gonioscopic examination starting from posterior to anterior is formed by:

- Iris processes
- Ciliary band
- Scleral spur
- Trabecular meshwork
- Schwalbe's line

Grading of Angle Structures

To assess glaucomatous or potentially glaucomatous eyes, grading of angle width plays a critical role. Its aim is to evaluate the status of angle, the degree of closure and risk of future closure. Currently Shaffer's, Schie's and Spaeth's grading systems are in use.

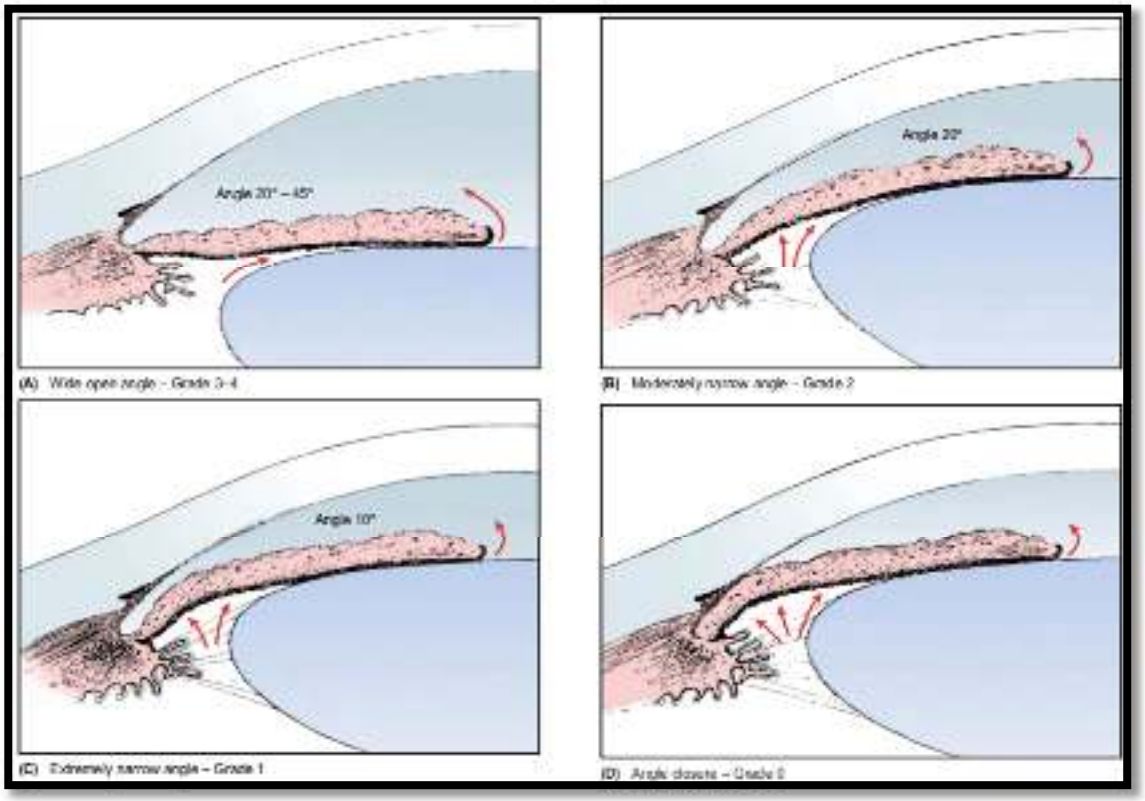


Fig 4- Grading of AC angle.

SHAFFER'S GRADING SYSTEM

SHAFFER'S GRADING SYSTEM			
Angle grade	**Degrees**	**Numeric grade**	**Clinical interpretation**
Wide-open angle	30-40	3-4	Closure impossible
Narrow angle (moderate)	20	2	Closure possible
Narrow angle (extreme)	10	1	Eventual closure probable
Slit angle	<10	S	Portions appear closed
Closed angle	-	0	Closure present

CLASSIFICATION OF GLAUCOMAS (4)

1. Open angle glaucoma:
    a. Primary open angle glaucomas
        i. IOP higher than normal range
        ii. Low tension glaucoma
    b. Secondary open angle glaucomas
        i. Pigmentary glaucoma
        ii. Pseudoexfoliation glaucoma
        iii. Steroid glaucoma
        iv. Lens-induced glaucoma
        v. Post-cataract surgery
        vi. Post-traumatic
        vii. Associated with intraocular hemorrhage
        viii. Associated with retinal detachment
        ix. After vitrectomy
        x. With uveitis

xi. With intraocular tumors

xii. Amyloidosis

xiii. Increased episcleral venous pressure

2. Angle closure glaucoma

   a. Primary angle closure disease

      On the basis of natural history :

      i. Primary angle closure suspect

      ii. Primary angle closure

      iii. Primary angle-closure glaucoma

      On the basis of mechanisms of anterior segment closure :

      i. Pupillary block

      ii. Plateau iris syndrome

      iii. Phaco-morphic block

   b. Secondary angle closure disease

      By 'Anterior pulling' mechanisms:

      Iris is pulled anteriorly by the contracting membrane or by peripheral anterior synechiae

      By 'Posterior pushing' mechanism:

      Iris is pushed anteriorly by posterior segment pathology. Ciliary body rotates forwards and the lens comes front.

3. Developmental glaucoma

   a. Primary congenital (infantile) glaucoma

   b. Secondary congenital glaucoma

SURGICAL MANAGEMENT OF GLAUCOMA

HISTORY

William Mackenzie (1830) first suggested sclerotomy and later proposed paracentesis (1854) in chronic stages of disease.Von Graefe announced the effect of basal iridectomy in relieving raised tension followed by Louis De Wecker (1869-71) who devised an anterior sclerotomy so as to increase the aqueous drainage by formation of failing cicatrix. Leopold Heine introduced cyclodialysis in 1900. Cyclodestruction was recommended in 1932.

To lower the IOP surgically, two approaches can be employed: 1. increase aqueous outflow and decrease aqueous inflow.[8] Most of the surgical techniques were proposed in the mid-1900s. Felix Lagrange introduced the initial filtering surgery of sclerecto iridectomy. Trabeculectomy was originally described in 1968[19] by Cairns, modified by Watson in 1972 performed by lifting a conjunctival flap, dissection of a partial thickness scleral flap followed by short length excision of Schlemm's canal with its trabecular adnexae.[20] It is now considered the gold standard filtering surgery.

TRABECULECTOMY

Trabeculectomy is creation of a surgical fistula draining aqueous from the AC into the sub-conjunctival space. The aqueous is then either absorbed into conjunctival blood vessels or lymphatics, or in case of a thin walled bleb, it passes straight through the conjunctiva into the tear layer.[4] Various modifications and supplementation have been introduced over the years to reduce complications and also improve postoperative outcomes.[21]

Modifications of Trabeculectomy

Some of the modifications include use of antimetabolites such as mitomycin C (MMC)[22] & 5-fluorouracil (5-FU)[23] to decrease the likelihood of bleb failure, and creation of a fornix-based rather than the traditional limbus-based conjunctival flap.

Of late "Ab interno trabeculectomy" using devices such as trabectome or kahook dual blade, iStent and Ex-PRESS shunt, CyPass Micro-Stent, XEN Gel Stent and Gonioscopy-assisted transluminal trabeculotomy are minimally invasive surgeries that increase trabecular outflow and effectively control IOP while maintaining a good safety profile.

Wound healing in Trabeculectomy

A bleb, like other tissues undergoes 4 stages of wound healing postoperatively:

1. ***Coagulative phase***

Incised vasculature causes an immediate leakage of plasma proteins, blood cells, and platelets. Activated platelets release a variety of chemicals, growth factors and clotting factors, all of which play a pivotal role in platelet aggregation and clot formation.

2. ***Inflammatory phase***

This phase occurs in the initial postoperative days, characterized by recruitment of inflammatory cells like neutrophils, monocytes differentiating into macrophages with the release of cytokines triggering the onset of proliferative phase.

3. Proliferative phase

Involves the generation of a new matrix occurring through angiogenesis and fibroplasia within a few days of surgery. This encompasses the formation of a granulation tissue (young fibroblastic connective tissue) lining the fistula by day 3-day 10.

4. Remodelling phase

In a few weeks, activated fibroblasts differentiate into myofibroblasts, a more contractile cell phenotype. There is simultaneous collagen production and degradation (mainly by Matrix metalloproteinases) from the tissue deciding the amount of scarring. Fibroblast cell death by apoptosis and partial resorption of blood vessels occur ultimately leaving behind a mature collagenous scar.[24–26]

Based on degree of filtration, a bleb(subconjunctival accumulation of aqueous) is classified as follows:[27]

Type I- Good filtration characterized by low IOP and a thin polycystic bleb

Type II- Good filtration characterized by low IOP and a shallow diffuse thin walled bleb

Type III- Poor filtration characterized by increasing IOP and a flat bleb secondary to episcleral fibrosis

Type IV- Poor filtration characterized by increasing IOP and an encapsulated bleb

A study showed that the most frequent reason for failure after trabeculectomy is due to fibrosis in episcleral and sub-conjunctival space. Wound healing modulation

can thus optimize the success rate of trabeculectomy by delaying the wound healing process.(28)

Wound modulators in Trabeculectomy

Modulation in wound healing is done to moderate the healing via pharmacological agents used intra operatively or post-operatively. Rudimentarily, it comprises of beta irradiation, topical corticosteroids. Other agents include antimetabolites such as MMC[29] or 5-FU[23] applied sub tenon's and/or sub-scleral flap. There is a delicate balance between optimal conjunctival healing and the suppression of scar formation in the filtering bleb.[30,31] The benefits provided by these modulators, also come with a unique set of complications which has thus led to the advent of other novel therapies.[8]

Beta irradiation

Cohen[32] suggested the use of beta irradiation at the surgical site in black patients who have a higher risk of surgical failure post-operatively. It was abandoned due to complications like necrosis of conjunctiva or sclera and lenticular opacities.

Corticosteroids

Sugar[33] described the possible effects of postoperative topical corticosteroids on filtration blebs. Some surgeons inject corticosteroids sub-conjunctivally away from the surgical site. Tissue culture studies have shown that corticosteroids inhibit cell attachment and proliferation.[34]

While the preliminary reports on the use of corticosteroids in IOP control was promising, but bleb failure in the long term continued to occur. This led to the arrival of additional wound modulators.

Anti-VEGF (Vascular endothelial growth factor)

Angiogenesis is an elemental factor in the proliferative phase of wound healing. Recombinant humanized monoclonal antibodies such as Bevacizumab and Ranibizumab targeting this aspect has been used as an adjunct to trabeculectomy. It has shown favourable initial results.[35–37]

5-Fluorouracil (5-FU)

Gressel et al.[38] conducted an experimental study on owl monkeys. Postoperative subconjunctival injections of 5-FU was given to one eye versus saline injection to the fellow eye in a randomized manner. Six of the eight eyes that received 5-FU demonstrated filtering blebs at 4th month while all the control eyes showed bleb failure within post-operative week 2.

It is a pyrimidine analogue inhibits thymidylate synthase and thus blocks DNA synthesis. This results in an arrest of proliferation of fibroblasts.[39,40] 5-FU can be given post-operatively as subconjunctival injections at a dose of 5mg BD for 7 days or as an intra-operative application during trabeculectomy at a dose of 25 mg per mL for five minutes after exposure of the sclera.[23] The reported complications of 5-FU are corneal epithelial toxicity and defect [41,42] and bleb leaks.[43]

Mitomycin C (MMC)

Chen et al.[44] demonstrated enhanced bleb survival with MMC. MMC is an alkylating agent developed from the bacterium 'Streptomyces caespitosus' which exerts its effect by interfering with DNA cross linking, mitosis and protein synthesis.[45] a survey conducted in 2016 stated that intraoperative application of MMC using cellulose-soaked sponges of 0.4mg/ml with a range of 0.2-0.5mg/ml for

2-2.5 mins with a range of 45 seconds to 4.5 minutes was the most acceped dosage for primary trabeculectomy.[46] It is applied between the conjunctiva and sclera, either before or after the scleral flap dissection, or under the scleral flap.[31,47]

MMC is associated with sustained cytotoxicity leading to loss of endothelial cells, cataract, hyphaema, flat AC & hypotony, suprachoroidal haemorrhage, hypotonus maculopathy, malignant glaucoma, blebitis, intraocular infections and endophthalmitis.[9,45,48,49]

The search for a more biological substance as a bleb modifier while simultaneously lowering the complications has brought us to the deliberation of the application of amniotic membrane in trabeculectomy.

Human amniotic membrane

Amniotic membrane is the inner layer of the fetal membranes. It is composed of an inner layer of epithelial cells, sitting on a basement membrane that is then connected to a connective tissue membrane by filamentous strands.[50] Rotth[51] and Sorsby[52] first suggested the use of amniotic membrane in ophthalmology in ocular surface disorders.

TGF-β is a potent stimulant of scarring, and a crucial element in wound healing, specifically in conjunctival fibrosis. It has been found in human aqueous and seems to play a role in healing post filtering surgery. Hence, inhibition of TGF-β appears to be a more physiologic approach to tackle wound healing modulation.

Amniotic membrane transplantation hinders fibrosis by down regulating transforming growth factor β (TGF β) signalling and myofibroblast differentiation.[10,53] It has proven anti-angiogenic and anti-inflammatory properties. It

provides a framework and a basement membrane over which epithelisation can occur and integrate well with the surrounding tissues favouring the formation of a filtering bleb. It facilitates a muted healing response, poor immunogenicity, and yet maintains a high hydraulic conductivity.[9,49,54,55]

There are various protocols to procure and store amniotic membrane. Kim et al recommended the following: the placenta is first thoroughly washed under sterile precautions with a mixture of balanced salt solution (BSS) and a combination of antibiotics (50 mg/ml penicillin, 50 µg/ml streptomycin, 100 mg/ml of neomycin & 2.5 mg/ml of amphotericin B). By blunt dissection amnion is separated from chorion and cut into multiple pieces of various sizes and placed on nitrocellulose sheets with the glistening epithelial side facing up. It is stored after cryopreservation, frozen at -80 degrees in Dulbecco Modified Eagles Medium/glycerol (1:1) until further use.[56]

Initially, the drawbacks observed postoperatively maybe hematoma formation beneath the membrane. The blood typically gets absorbed or if excessive, may need drainage, by creating a minute opening in the transplanted membrane. Degradation of this graft prematurely and cheese wiring may occur, demanding frequent repeat transplantations.[56,57] The incidence of post –amniotic membrane transplantation infections is only about 1.6%. Gram-positive bacteria are generally the common isolates.[12]

The rationale behind conducting this prospective interventional longitudnal study was to evaluate the safety and effectiveness of amniotic membrane transplantation in trabeculectomy for managing glaucoma.

Fig 5a- Human amniotic membrane on nitrocellulose paper

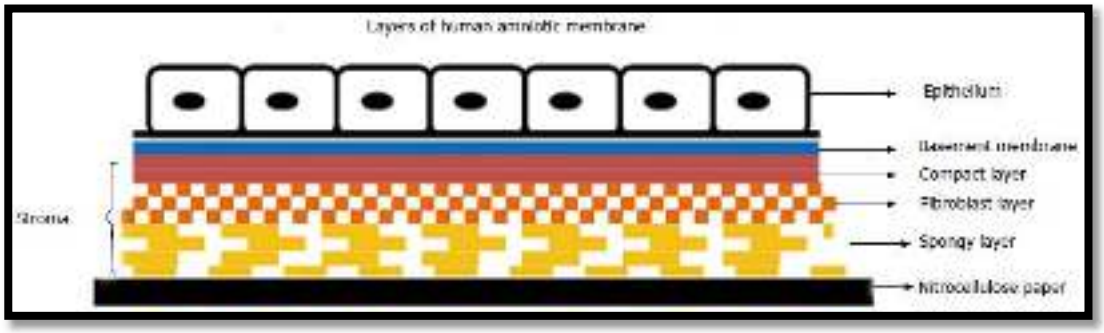


Fig 5b- Schematic representation of layers of human amniotic membrane on nitrocellulose paper

SURGICAL STEPS IN TRABECULECTOMY

Aseptic precautions & Anaesthesia:

Peribulbar block is given with lignocaine hydrochloride 2% and bupivacaine 0.5% under aseptic precautions as follows. Skin over eyelids, forehead over half side of face and nasal bridge is painted with 5% povidone iodine solution. Conjunctival cul-de-sac is irrigated with 1:10 povidone iodine solution. A universal speculum is inserted to keep the lids open.

Site of primary trabeculectomy is generally superonasal.

Tractional sutures

Tractional sutures are of two types:

- Superior rectus bridle suture: Suture (4-0 silk) is placed 10-15 mm behind the limbus. It holds the risk of hematoma formation, conjunctival perforation and release of growth factors leading to fibrosis at the site of the filtering bleb.[58]

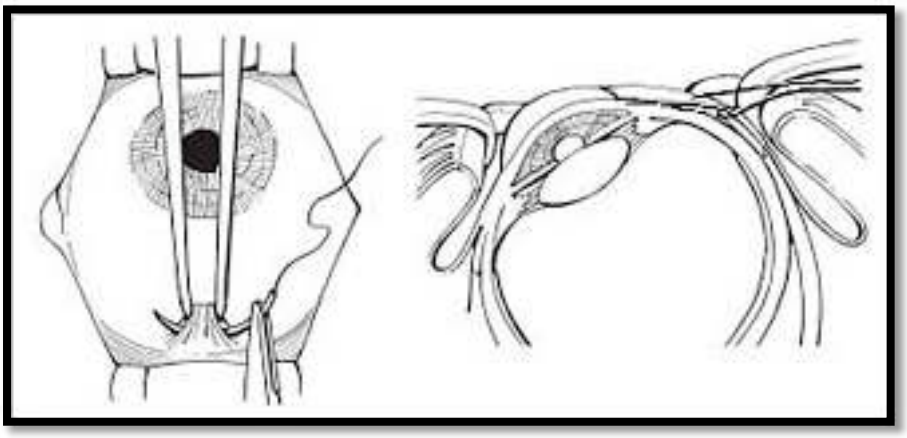


Figure 6a

- Clear corneal traction suture: Traction provided by this suture (using 7-0/8-0 vicryl) is superior to that of bridle suture. It carries the risk of corneal perforation if the depth of penetration is too deep and cheese wiring of corneal tissue in case of a superficial bite.

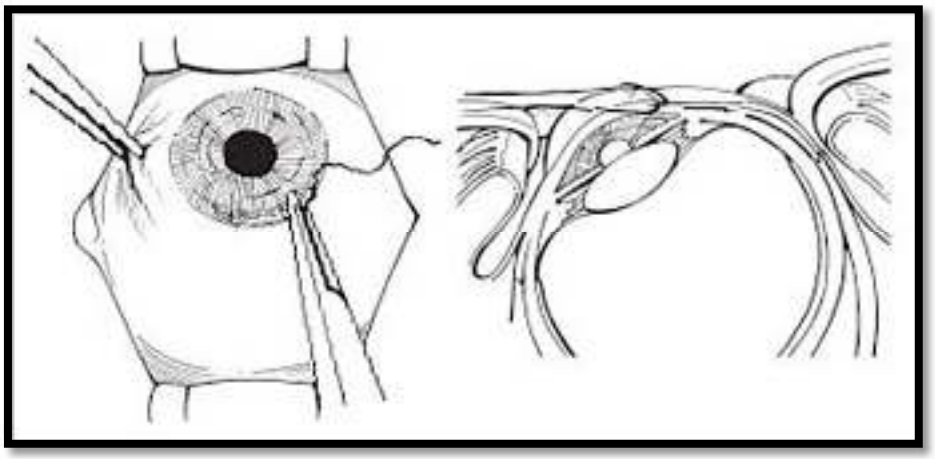


Figure 6b

A corneal traction suture is considered superior to the bridle suture, but the superior rectus bridle suture is technically easier.

Haemostasis

Thorough subconjunctival and episcleral haemostasis is achieved by wet field cautery. This is to attain adequate exposure for dissection and also to prevent postoperative scarring and failure of bleb.

Conjunctival incision and flap

The flap can be fornix-based or limbal-based.

- Limbal-based flap: Conjunctival incision is made deep in the fornix with base at the limbus and is reflected anteriorly to expose scleral bed.

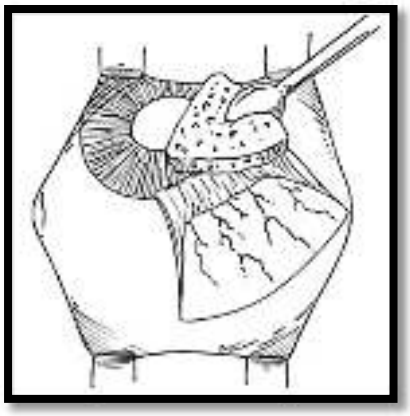


Figure 6c

- Fornix-based flap: Conjunctival incision along the limbus with base at fornix.

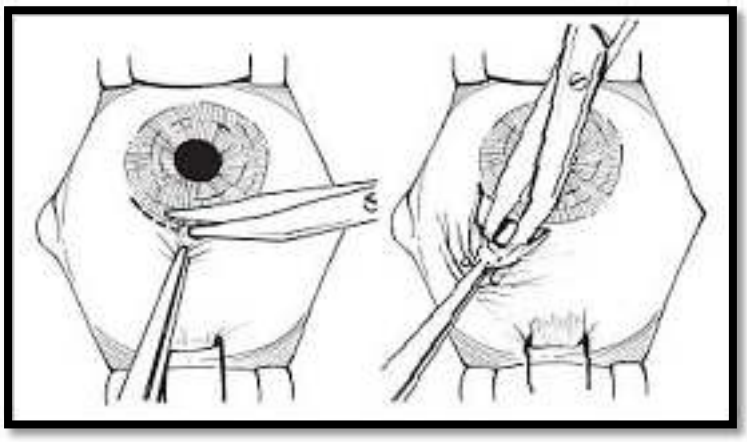


Figure 6d

Scleral flap dissection

A 4 x 4.5 / 4.5 x 5 mm rectangular or 4.5 to 5 x 3.5 mm triangular flap of partial thickness (ideally half the thickness of sclera) with its base resting at the limbus is dissected. The dissection is carried on till the blue grey barrier is crossed. Here, the white scleral fibres merge into the grey zone, overlying the scleral spur. The dissection is further proceeded on into 1mm of clear cornea. Before entry into the anterior chamber antimetabolites may be applied before forming the scleral flap or even after.

The scleral flap should neither be too thick as it may end up offering excessive resistance to aqueous flow, nor too thin resulting in a higher chance of flap dehiscence or aqueous leakage and hypotony.[59]

Paracentesis

After the scleral flap is secured, a paracentesis site is made at the peripheral limbus to fill the AC to be maintained with Ringer lactate solution or viscoelastic based on the need during surgery.[4]

Sclerostomy

After paracentesis, the inner sclerostomy can be performed just anterior to the scleral spur in one of two ways:

- A block of 1.5 - 2 x 3 mm, at the base of the scleral flap is cut with the Vannas scissors.
- A compact neat block of dimensions 1 -1.5 x 1.5-2 mm with no jagged margins is made using a Kelly Descemet's punch. This ensures controlled cutting always, thus avoiding shallowing of anterior chamber.[60]

A posterior removal of the block increases the risk of hemorrhage.[4]

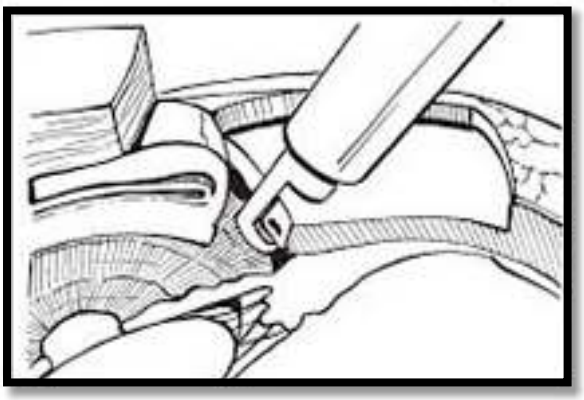


Figure 6e

Peripheral iridectomy (PI)

Through the sclerostomy site, PI is done with a Vannas scissors with the base being as large as the inner sclerostomy opening. A small number of studies have claimed that trabeculectomy possibly will function without a PI.[61] However, in the Indian scenario with the high prevalence of angle closure glaucoma, it would be rather judicious to perform a peripheral iridectomy in a trabeculectomy or a phacotrabeculectomy surgery.[60]

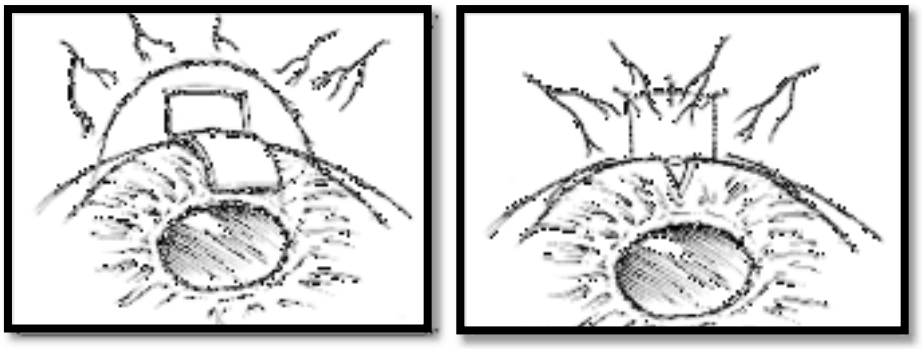


Figure 6f

Scleral flap closure

9-0 or 10-0 nylon sutures is used to approximate the scleral flap. AC is formed by injecting normal saline, and a slow fluid leakage through the scleral flap is looked for indicating adequate filtration. Flow adequacy can be tested with a surgical instrument by depressing or lightly 'burping' posterior to the scleral flap.(4)

Sutures applied to the scleral flap control aqueous outflow. Resistance to the aqueous outflow is mainly regulated by the approximation of the flap to the sclera beneath, adjoining the sclerostomy. This is in turn governed by the position of the sutures and their tension.

If sutures are too loose, aqueous flow will be too high, and may cause ocular hypotony. If the sutures are too tight, the intraocular pressure will stay too high, thus increasing the possibility of abrupt loss of the remaining field of vision ("snuff out") or added loss of ganglion cells with consequent worsening of glaucomatous damage to optic nerve head.(60)

Conjunctival flap closure

A water tight conjunctival closure is necessary for a functional postoperative bleb. This is performed using 10-0 nylon or 10-0 vicryl sutures inspecting the condition of anterior chamber and the bleb.[4]

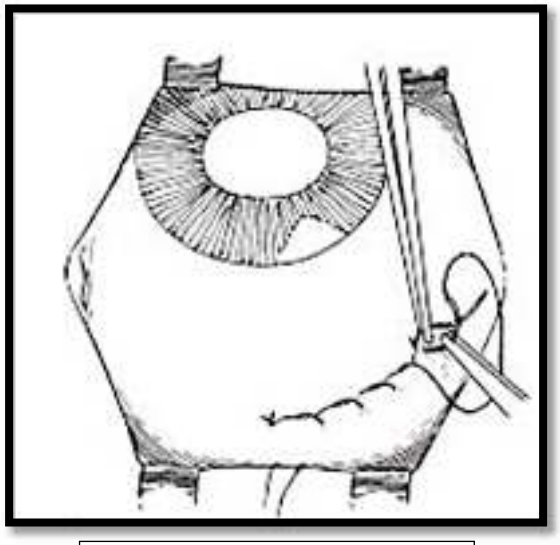


Figure 9g

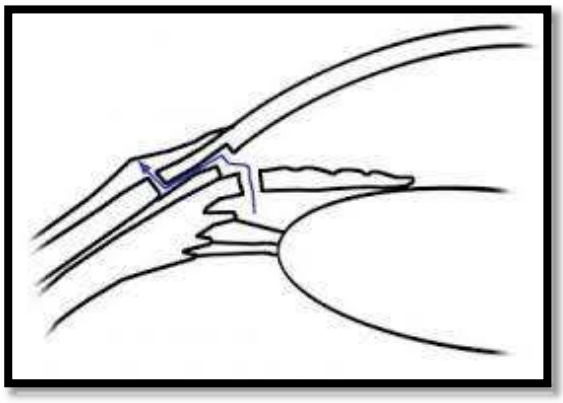


Fig 7- Schematic representation of aqueous outflow after filtering surgery

COMPLICATIONS OF TRABECULECTOMY

Intra-operative complications

1. Traction suture:

   Superior rectus bridle suture-

   - Superior rectus hematoma
   - Globe perforation
   - Severed superior rectus tendon
   - Subconjunctival haemorrhage

   Clear corneal traction suture-

   - Cheese wiring
   - Corneal perforation

2. Conjunctival flap:
   - Conjunctival buttonhole/tear
   - Subconjunctival hemorrhage

3. Scleral flap dissection:
   - Improper thickness of the flap making it staphylomatous/highly resistant to outflow.
   - Disinsertion of the superficial flap.
   - Incomplete removal of Descemet's membrane.

4. Sclerostomy:
   - Incomplete fistula formation
   - Corneal/Iris/Lens injury
   - Plugged sclerostomy site

5. Corneal injury:
   - Descemet's membrane detachment

6. Iridectomy:
    - Incomplete/Large iridectomy
    - Iris incarceration/prolapse
    - Anterior chamber hyphaema from iris bleed
    - Iridodialysis
7. Others:
    - Cyclodialysis
    - Vitreous loss
    - Shallow anterior chamber with/without hypotony
    - Conjunctival wound leak due to improper closure
    - Serous choroidal detachment
    - Expulsive choroidal haemorrhage
    - Retained visco-elastic material

Early Post-Operative Complications

1. Hyphema
2. Shallow anterior chamber
3. Hypotony
4. Bleb leak
5. Vitreous haemorrhage
6. Choroidal detachment
7. Suprachoroidal haemorrhage
8. Malignant glaucoma
9. Endophthalmitis
10. Sympathetic ophthalmia

Late Post-Operative Complications

1. Cataract
2. Decreased visual acuity
3. Encapsulated bleb
4. Progressive scarring of bleb
5. Thin walled blebs
6. Overfunctioning and diffuse blebs
7. Dellen
8. Hypotonous maculopathy
9. Bleb related infections
10. Endophthalmitis

BLEB MORPHOLOGY

Several methods have been proposed for classifying bleb morphology clinically:

A. *"***Moorfields Bleb Grading System**

Bleb is assessed either on slit lamp/photographically with respect to height and vascularity in three zones:

- Central bleb
- Peripheral bleb
- Non bleb

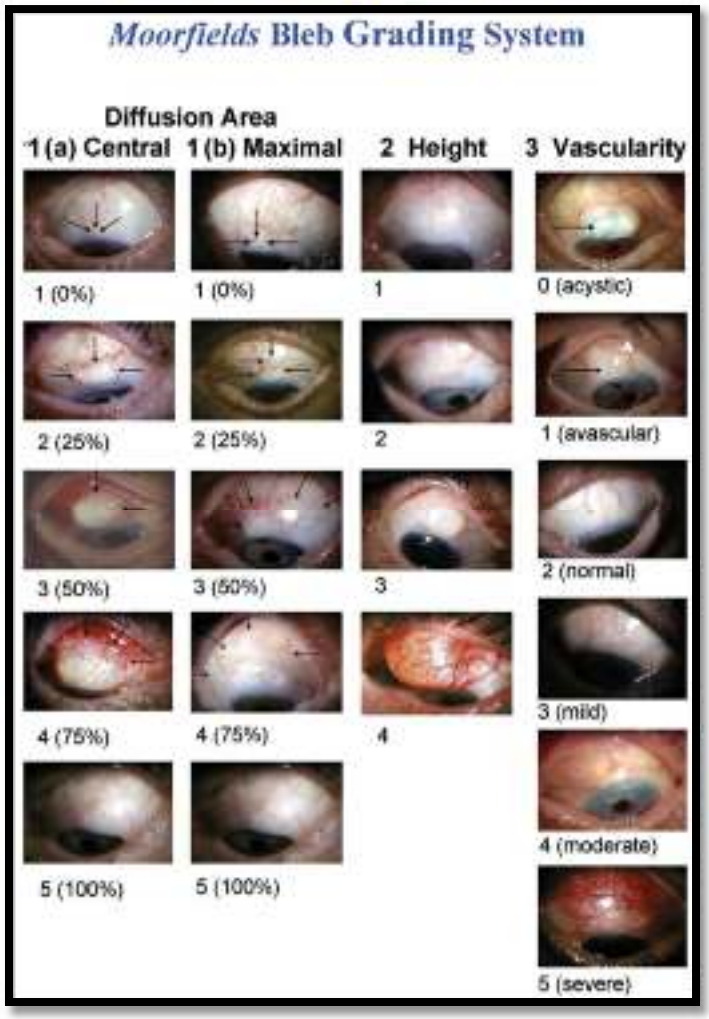


Fig 7- Moorfield's Bleb Grading System

1. Central bleb area: The central demarcated area relative to the superiorly visible conjunctival field is categorized into 5 percentages (0%, 25%, 50%, 75% and 100%)
2. Peripheral bleb area: Maximal area of bleb using a similar percentage scale i.e., the maximal diffusion area
3. Bleb height: Classified as flat, low, moderately elevated, or maximally elevated on slit lamp examination.
4. Vascularity: It is the most essential prognostic factor for bleb failure. It has five grades: avascular, normal, mild vascularity, moderate vascularity and severe vascularity. It is applicable to all three zones of the bleb."

B. "Indiana Bleb Grading System

The bleb is assessed for four main parameters either on slit lamp or photographically:

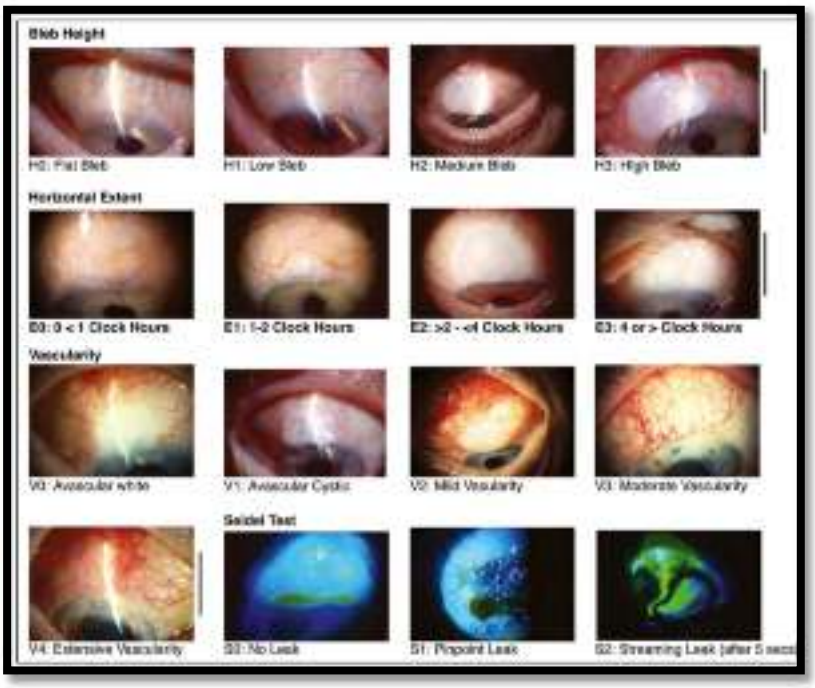


Fig 8- Indiana Bleb Grading System

1. Bleb height: Maximal vertical elevation of bleb-flat, low, medium or high.
2. Horizontal extent: Maximal horizontal extent of bleb according to clock hours comparable to limbal clock hours: >1 hr, 1-2 hr, >2-<4 hr and >4 hr.
3. Vascularity: It is defined as white and avascular, cystic and avascular (with microcysts), mild vascularity, moderate vascularity, and extensive vascularity.
4. Seidel leakage: Bleb leak tested with fluorescein strip and classified the bleb as: no leak, multiple pinpoint leaks without streaming, brisk streaming within 5 seconds."[4]

MATERIALS AND METHODS

The present study was conducted at the Department of Ophthalmology, KLES Dr.Prabhakar Kore Hospital & Medical Research Centre, Belagavi from 1st January 2020 to 31st December 2020 who are have been diagnosed to have Glaucoma to evaluate safety and efficacy of using amniotic membrane as an adjunct to primary trabeculectomy.

Source of Data

All patients who are ≥ 18 years of age and have been diagnosed with glaucoma and planned for trabeculectomy at the Department of Ophthalmology, KLES Dr.Prabhakar Kore Hospital & Medical Research Centre, Belagavi.

Method of collection of data

Study design: A one year prospective, longitudinal, interventional, hospital based study.

Study period: One year – 1st January 2020 to 31st December 2020.

Sample size: It is calculated according to the formula:

$$n = \frac{Z^2 P(1-P)}{d^2}$$

where n = sample size,

Z = Z statistic for a level of 95% confidence,

P = expected prevalence or proportion

(for glaucoma is 2.1%, P = 0.02), and

d = precision (5%, d = 0.05).

The sample size for this study is 31.

Selection criteria:

Inclusion criteria-

1. Individuals >/= 18 years of age.
2. Primary open angle glaucoma and no less than one of the following:

1. Inadequate control of target IOP with the use of topical antiglaucoma medications.
2. Progression of damage to optic nerve on two subsequent visual field testing and increase in cup-to-disk ratio over a period of 2 years
3. No previous intraocular surgery
4. Angle closure glaucoma
5. Normotensive glaucoma
6. Combined cases of cataract with glaucoma posted for phaco-trabeculectomy.

Exclusion criteria-

- Uncooperative behaviour i.e. inability to comprehend commands.
- Any other type of glaucoma(neovascular, pseudoexfoliation, pigment dispersion glaucoma)

- Coexistence of any ocular anterior segment disease presently or in the past (excluding cataract)
- Prior unsuccessful filtration surgeries.
- Previous laser procedures

Procedure:

1. After the approval from the institutional review board and ensuring that all the study procedures adhere to the tenets of the Declaration of Helsinki the study shall be carried out.
2. Patients who satisfy the mentioned criteria were enrolled in the study as subjects and demographic data such as name, age, gender, profession, and residing address were recorded on a pre-designed proforma at the time of the patient's first visit.
3. A written and informed consent was taken from all patients on describing the procedure and the risks associated with it.
4. After enrolment, patients will be undergoing the following surgical procedure: Trabeculectomy with amniotic membrane transplant
5. Human amniotic membranes of 3.5cm x 3.5cm were procured and stored at -80°C in sterile vials containing Dulbecco's medium and was allowed to defrost for 10 mins prior to the surgery at room temperature and rinsed twice with BSS before transplantation.

6. Prior to procedure the following investigations will be done :

    - A thorough history of the patient shall be noted and duly filled in the proforma.
    - The number and details of the anti-glaucoma medications used, will be recorded.
    - The best-corrected visual acuity shall be recorded with the help of Snellen's chart.
    - Refraction
    - Detailed Slit lamp examination will be done to exclude other ocular co-morbidities.
    - Fundus examination will be done using direct and indirect ophthalmoscopy. Glaucomatous optic nerve head changes are localized notching neuro-retinal rim (NRR) thinning, alpha and beta zone, bayonetting, laminar dot sign or retinal nerve fibre layer defects.
    - IOP will be measured with a Non-Contact or Applanation Tonometer.
    - Gonioscopy shall be done using a Goldmann 3 mirror gonioscope and shall be graded according to Shaffer's grading.
    - Visual-Field assessment shall be done. Defects in Visual field shall be deemed as glaucomatous: when at least 2 of the 3 Anderson's criteria "≥ 3 non-edge points in a cluster depressed to $P < 5\%$ and 1 of which is depressed to $P < 1\%$, Glaucoma Hemifield Test outside normal

limits and Pattern Standard Deviation depressed to $P < 5\%$" are fulfilled.

- Photographic records were maintained

7. On diagnosing glaucoma, the patient was started on medical management of glaucoma with one or more anti-glaucoma drugs in combination.
8. The decision for surgery was made based on the following criteria:

   i. Patient with uncontrolled IOP

   ii. Patient with visual field progression

   iii. Patients with poor compliance with medical therapy

   iv. Patients who couldn't afford the medical management

   v. Poor follow-up and regularity

Pre-operative steps:

1. Lacrimal sac syringing is done to confirm patency of nasolacrimal passages..

2. Pulse Rate, Blood Pressure, Random Blood Sugar levels will be assessed.

1. Antibiotic drops will be instilled in the patient's eyes a day before the surgery.

2. Oral antibiotics were started Tab. Ciprofloxacin 500mg twice a day/ Tab. Levofloxacin 500mg once a day and/or IOP lowering drug i.e., Tab. Acetazolamide 250mg twice a day.

3. A day before the surgery IV Mannitol 20%, 200ml over 20 minutes was given to the patient along with the measurement of pre-mannitol and post-mannitol IOP.

4. On the day of the surgery, before the procedure, IV Mannitol 20%, 200ml over 20 minutes was given to the patient along with the measurement of pre-mannitol and post-mannitol IOP.

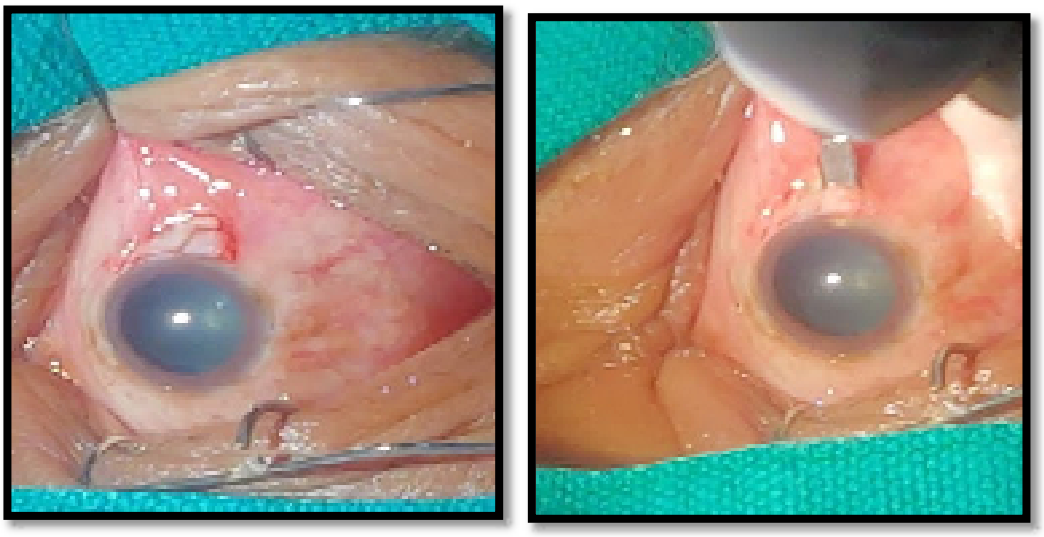
Anaesthesia:

Surgery was performed by one surgeon under peribulbar anesthesia consisting of 5ml lignocaine hydrochloride 2% with 1500 units of Hyaluronidase, without adrenaline and 5ml bupivacaine. Post-block IOP was recorded.

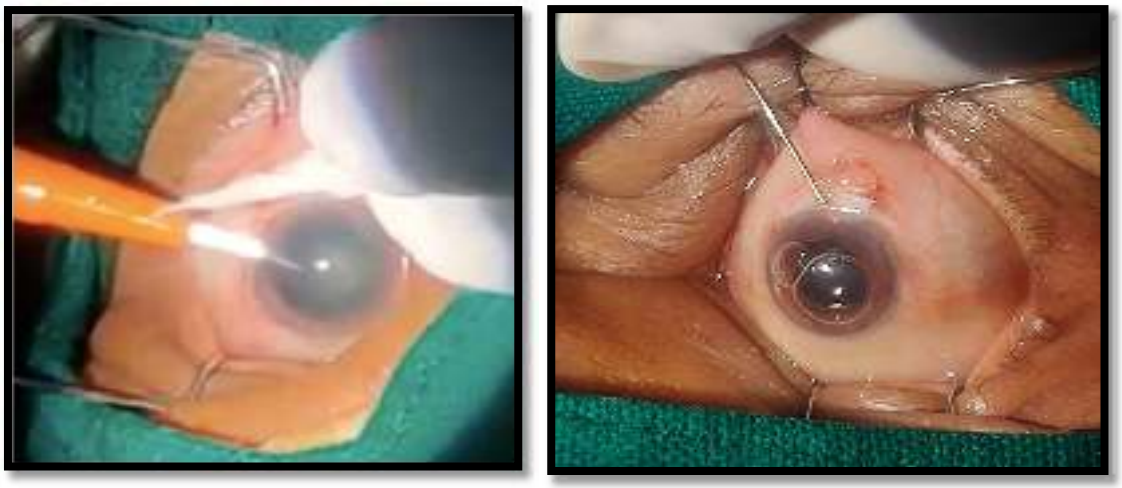
Steps of surgery:

- The eye was painted with povidone-iodine and draped, universal eye speculum put.
- Superior rectus bridle suture was taken with 4-0 silk.
- Fornix-based conjunctival flap at the superior limbus was raised and hemostasis achieved with wet field cautery.
- A fornix-based conjunctival flap was raised superiorly followed by wet-field cauterization and removal of Tenon's from the scleral surface.

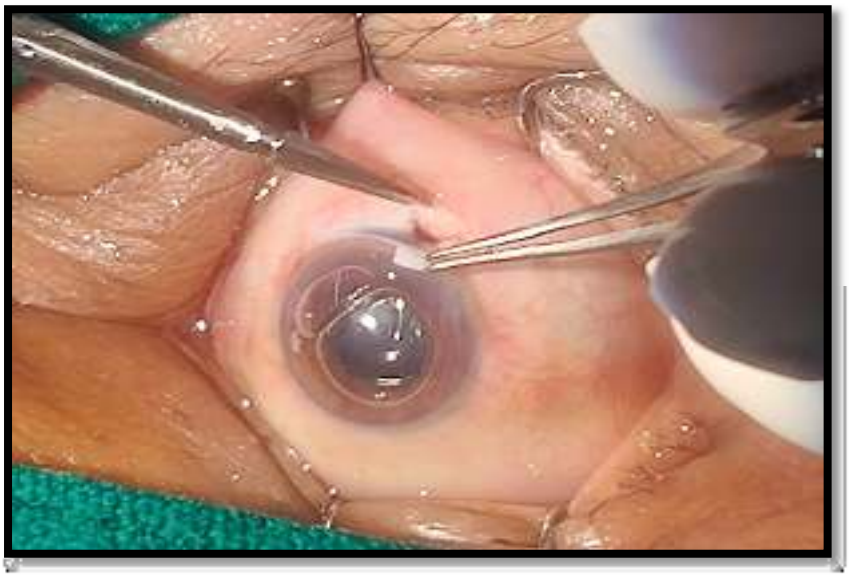
- A 3X4mm partial thickness scleral flap was dissected and if a combined procedure was planned then a linear incision adjacent to it 2mm from the limbus.



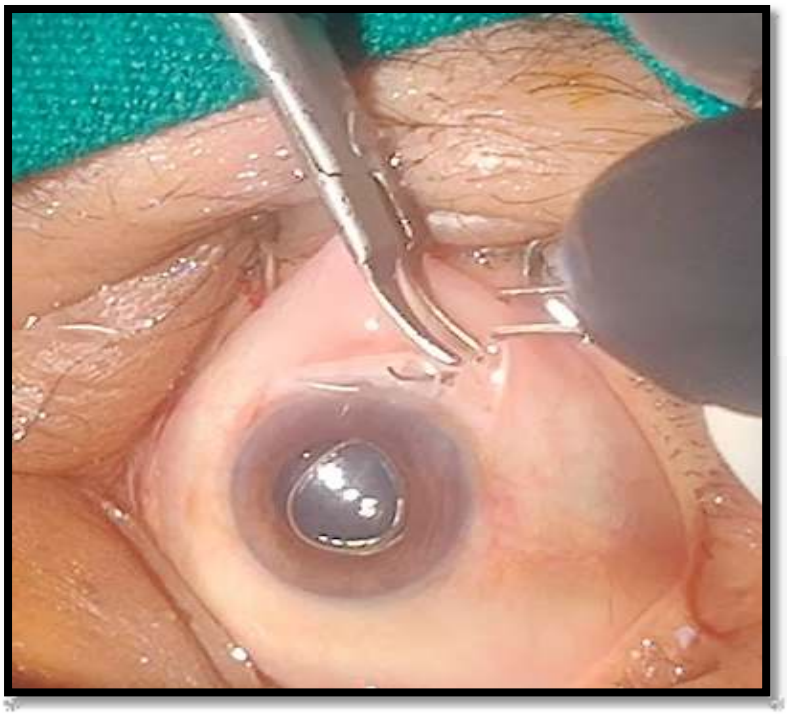
- If combined surgery was planned then lens extraction was performed and anterior chamber was maintained with air after paracentesis.



- A sclerostomy was accomplished with the help of Vanna's scissors/ Kelly's Punch, and PI was done with iris scissors/Vanna's scissors.

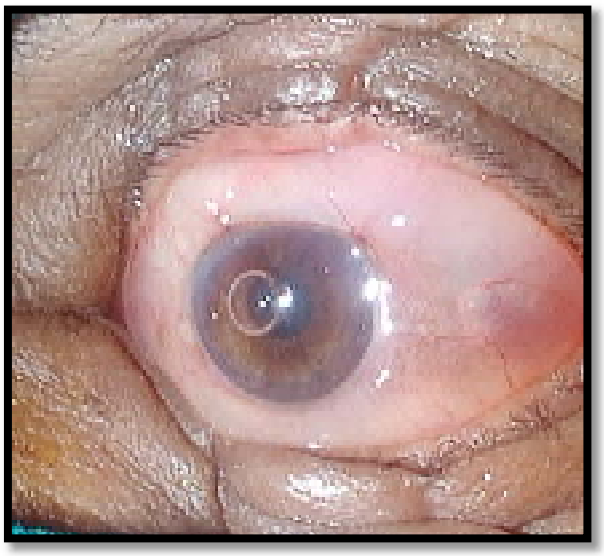


- The scleral flap sutured using two 10-0 nylon sutures.



- Once effective filtration was achieved, amniotic membrane of 4X4 mm was cut and placed above the scleral flap such that the spongy layer is towards the sclera. The graft was not sutured.

- The conjunctival flap raised earlier was repositioned with the membrane beneath and interrupted 8-0 Vicryl sutures were taken. Precautions should be taken so as to not fold the membrane while suturing.



- BSS was injected via the paracentesis port into the AC to ensure flow of aqueous through the newly fashioned bleb & to check for any leaks.

- Dexamethasone 0.5cc & Gentamicin 0.5cc was injected subconjunctivally along with atropine 2% eyedrops/ointment and antibiotic eye ointment was administered.

Postoperative steps:

1. Postoperatively, next morning the eye patch was removed & the patient was examined on day1, week 1, week 2, 1st month, 2nd month, 3rd month.

2. Patients were started on topical antibiotic eye drops and steroid combination eye drops 8 times per day for 2 weeks and then tapered over 6 weeks & homatropine 1% eye drops 2 times a day for 2 to 3 weeks.

Follow up:

1. At each visit the following parameters shall be assessed:

   a. Visual acuity:

      i. Uncorrected

      ii. Best corrected

   b. Postoperative IOP

   c. Slit-lamp examination of anterior segment and bleb morphology i.e., height, central and peripheral area, vascularity on the basis of 'Moorfield's Bleb Grading System' was performed on every visit.

Outcome:

The outcomes are defined as follows:

A. Complete success: Postoperative IOP of 21mmHg or less and a reduction in pre-operative IOP of 20% or greater without anti-glaucoma medication.

B. Qualified success: Postoperative IOP of 21mmHg or less and a reduction in pre-operative of 20% or greater without anti-glaucoma medication.

C. Failed surgery: Postoperative IOP greater than 21mmHg or a reduction in pre-operative of less than 20% even with anti-glaucoma medication or the need for additional surgery including needling or bleb revision.

D. Total success: Both complete and qualified success cases.

OBSERVATION AND RESULTS

The present study was conducted at the Department of Ophthalmology, KLES Dr.Prabhakar Kore Hospital and Medical Research Centre, Belagavi during the study period, from 1st January 2020 to 31st December 2020.

Thirty one patients who are >18 years of age and have been diagnosed with glaucoma and planned for trabeculectomy were enrolled in this study. In all thirty one eyes, amniotic membrane transplantation was performed. Out of the thirty one patients, one patient was lost for follow up due to the COVID-19 pandemic and thus excluded from the study.

Table 1: Age wise distribution of patients

Age (in years)	No. of cases	Percentage (%)
51-60	8	26.67%
61-70	14	46.67%
71-80	6	20%
81-90	2	6.67%
Mean ± SD	65.17 ± 8.23	

In our study, the age ranges from 50 to 82 years with mean age 65.17 ± 8.23 years. Out of the 31 patients, majority of the patients (14 patients i.e., 46.67%) were between 61- 70 years, 8 patients (26.67%) belonged to 51-60 years and 6 patients (20%) belonged to 71-80 years. Only 2 patients (6.67%) were between the ages 81-90 years. No patients were below 50 years of age in our study.

Graph 1: Age wise distribution of patients

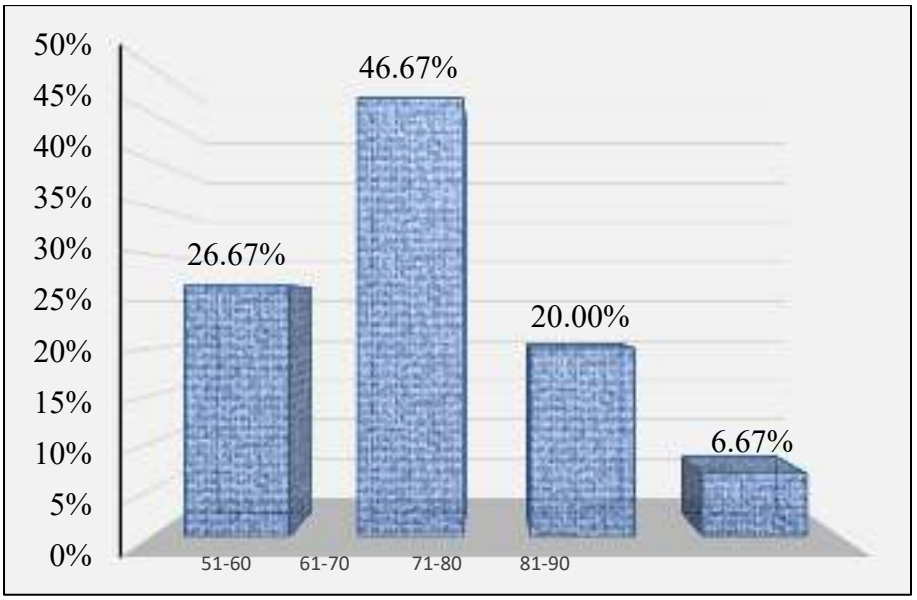


Table 2: Gender distribution among patients

Gender	No. of cases (n=30)	Percentage
Female	5	16.67%
Male	25	83.33%

Out of 30 patients in this study, majority were males i.e., 25 (83.33%) were males and 5 (16.67%) were females. This gives a male to female ratio of 5:1, indicating a higher male predisposition.

Graph 2: Gender distribution among patients

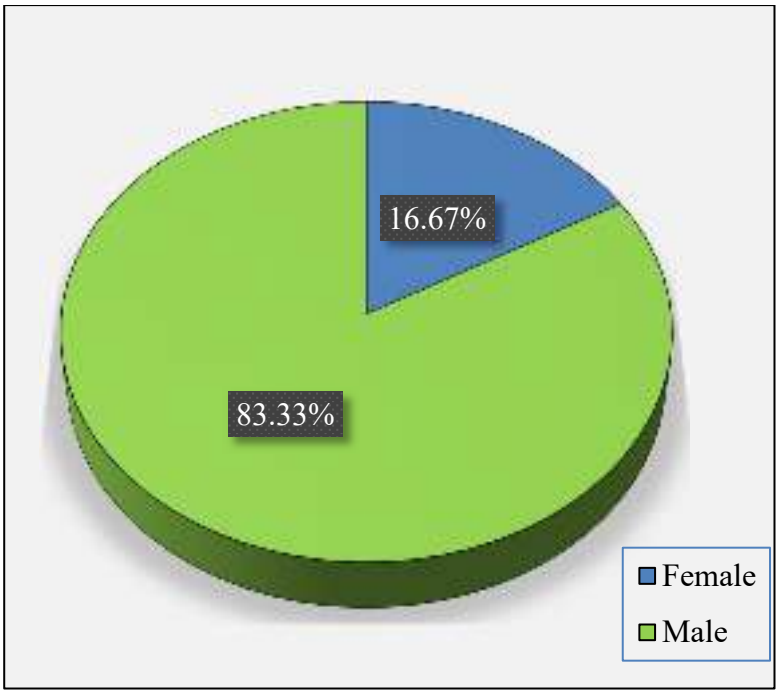


Table 3: Types of glaucoma with age and gender among patients

Variable	Sub-Category	Diagnosis / Type of Glaucoma				p-value
		Normotensive (NTG)	Primary angle closure (PACG)	Primary open angle (POAG)	Total	
Age (years)	51-60	0	1 (100%)	7 (29.17%)	8	0.0745[MC]
	61-70	2 (40%)	0	12 (50%)	14	
	71-80	1 (20%)	0	5 (20.83%)	6	
	81-90	2 (40%)	0	0	2	
					30	
Gender	Female	2 (40%)	0	3 (12.5%)	5	0.3298[MC]
	Male	3 (60%)	1 (100%)	21 (87.5%)	25	
					30	

Abbreviation: MC – Chi square test with Monte Carlo simulation.

Patients who were diagnosed with primary open angle glaucoma i.e. 24 (80%), majority of them i.e. 12 (50%) were in an age range of 61-70 years. 5 (16.67%) were diagnosed with normotensive glaucoma with 2 patients (40%) in both age ranges of 61-70 years and 81-90 years.1 (3.33%) has primary angle closure glaucoma with an age range of 51-60 years. Most of the male cases suffered from POAG, 21 (87.5%) and 3 (12.5%) were females out of 30 cases.

From Chi square test, we observe that, there is no significant association of age, gender and diagnosis/type of glaucoma.

Graph 3: Types of glaucoma with gender

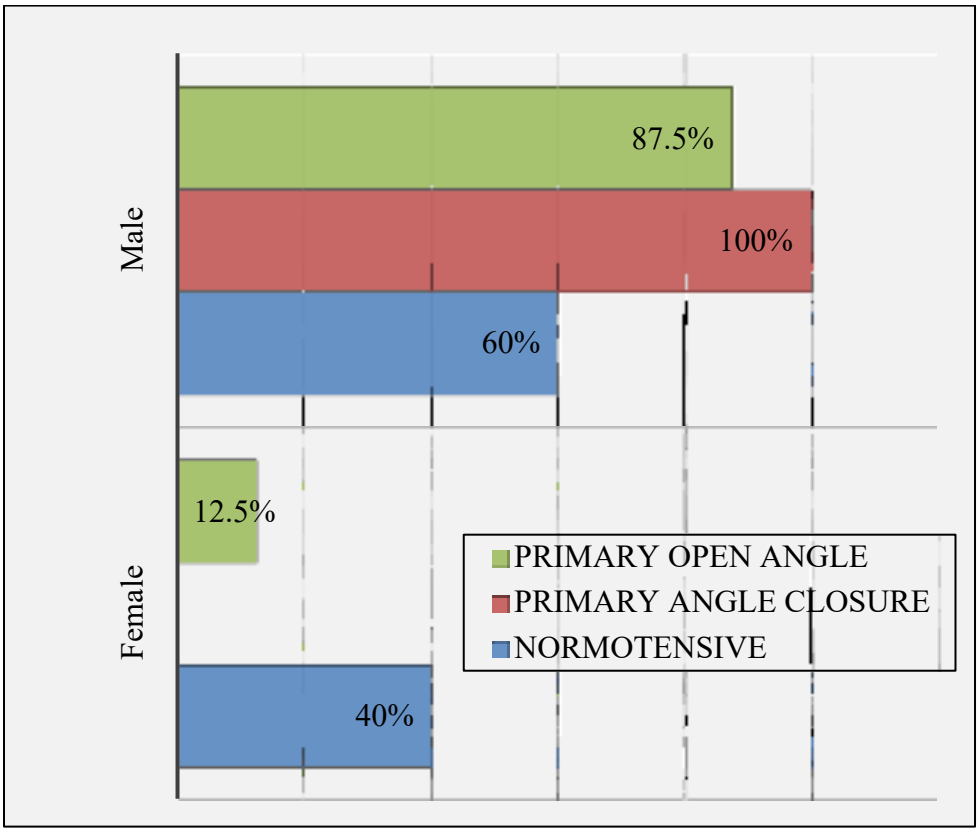


Table 4: Co-morbidities and correlation with the type of glaucoma

Co-morbidities	Diagnosis / Type of Glaucoma			P-value
	Normotensive (NTG)	Primary angle closure (PACG)	Primary open angle (POAG)	
DM	1 (20%)	1 (100%)	1 (4.17%)	0.4583[MC]
HTN	1 (20%)	0	7 (29.17%)	
IHD	0	0	1 (4.17%)	
Both IHD & HTN	0	0	2 (8.33%)	
Nil	3 (60%)	0	13 (54.17%)	

Abbreviation: MC – Chi square test with Monte Carlo simulation.

Out of 30 patients, majority of the patients i.e. 13 (54.17%) that are diagnosed with POAG have no co-morbidities while 7 (29.17%) have hypertension (HTN), one (4.17%) has diabetes mellitus (DM) and Ischemic heart disease (IHD). 2 patients (8.33%) having POAG have both IHD and HTN. Only a minority of patients with co-morbidities have been diagnosed with NTG and PACG.

From Chi square test, we observe that, there is no significant association of comorbidity and diagnosis/type of glaucoma.

Graph 4: Distribution of comorbidity with type of glaucoma

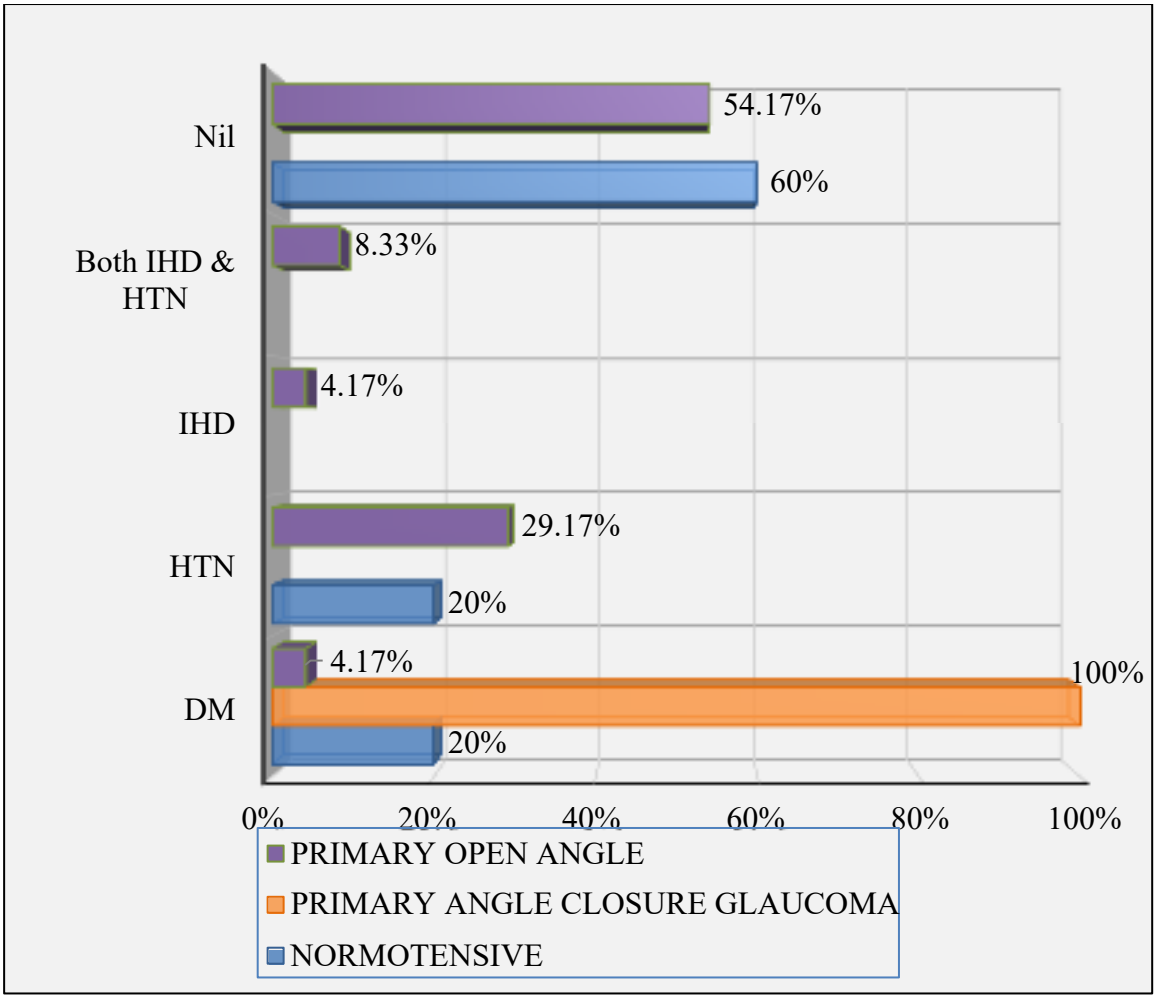


Table 5: Distribution of type of surgery

Type of surgery	No. of patients (n = 30)	Percentage (%)
Trabeculectomy	4	13.33%
Combined trabeculectomy	26	86.67%

Only trabeculectomy with amniotic membrane transplantation was performed on 4 (13%) eyes. However, combined procedure i.e. lens extraction with trabeculectomy was done on 26 (86.67%) eyes. The choice of surgery was made based on the need for cataract extraction according to the grade of cataract.

Graph 5: Distribution of type of surgery

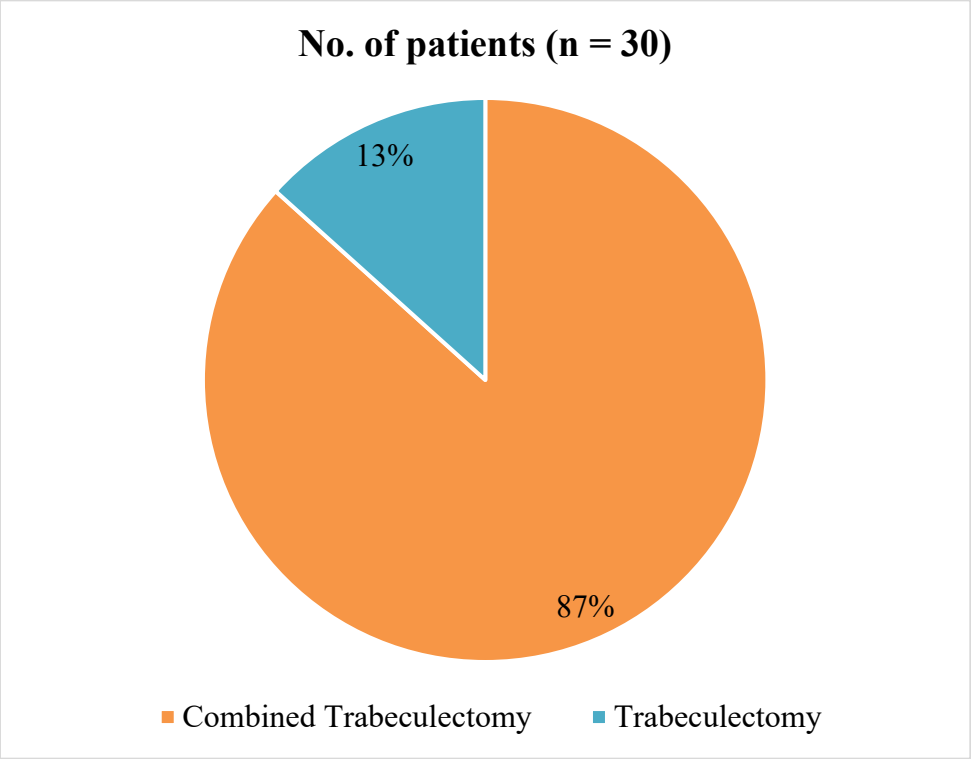


Table 6: IOP Reduction from pre-operative IOP to post-operative follow-up visits

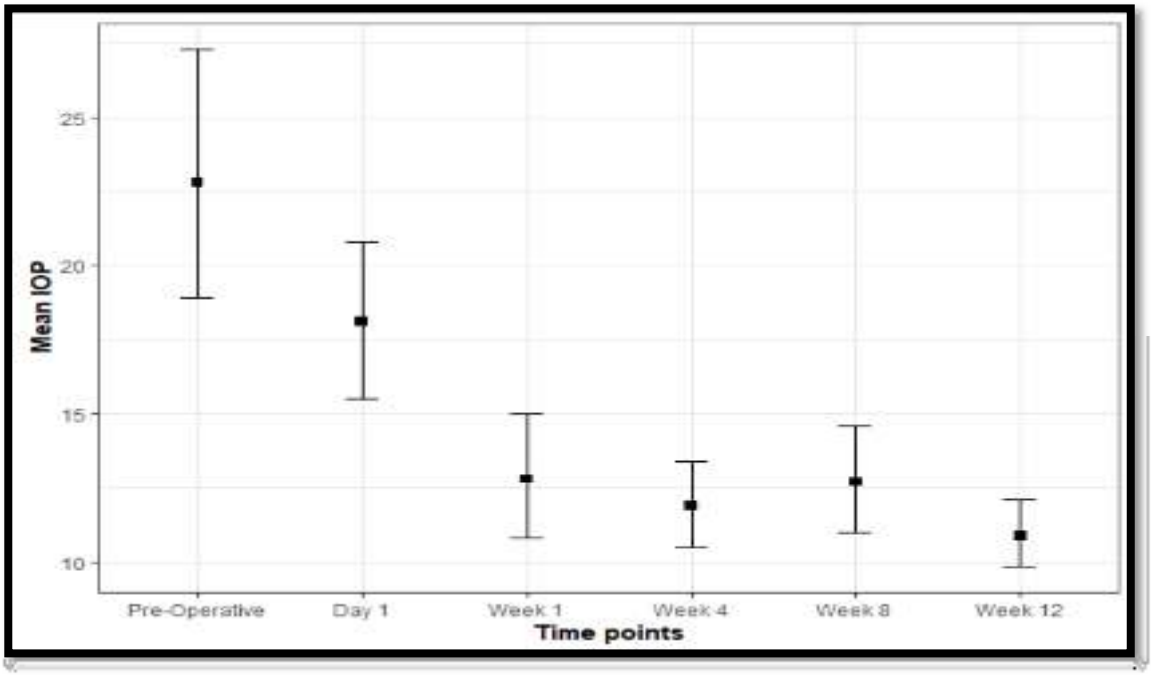
Time points	IOP (in mm Hg) Mean ± SD	Percentage reduction from Pre-op IOP (Mean ± SD %)	P-value
Pre-Operative	22.8 ± 12.04		**< 0.001***
Day 1	18.1 ± 7.4	6.71 ± 52.91	
Week 1	12.8 ± 6.01	35.24 ± 35.25	
1 month	11.93 ± 4.17	38.77 ± 28.68	
2 months	12.68 ± 5	35.23 ± 30.22	
3 months	10.93 ± 3.16	43.3 ± 23.65	

*Abbreviation: * indicates statistical significance.*

Pre-operatively the IOP was 22.8 ± 12.04 mm Hg. On day 1 post-operatively there was a 6.71 ± 52.91 % reduction in the IOP to 18.1 ± 7.4 mm Hg. One week later, the reduction was, 35.24 ± 35.25 % to 12.8 ± 6.01 mm Hg. By 3 months the IOP fell by 43.3 ± 23.65% to a mean of 10.93 ± 3.16 mmHg.

From one way repeated measures of ANOVA, we observe that, there is significant difference in IOP over the time points. From post hoc analysis, it is observed that pre-operative IOP is significantly different from IOP at 1st week, 1st month, 2nd month and 3rd month. IOP at 1st day is significantly different from IOP at 1st week, 1st month, 2nd month and 3rd month.

Graph 6a: IOP Reduction from pre-operative IOP to post-operative follow-up visits



Graph 6b: Comparison of pre-operative IOP and 3rd month IOP

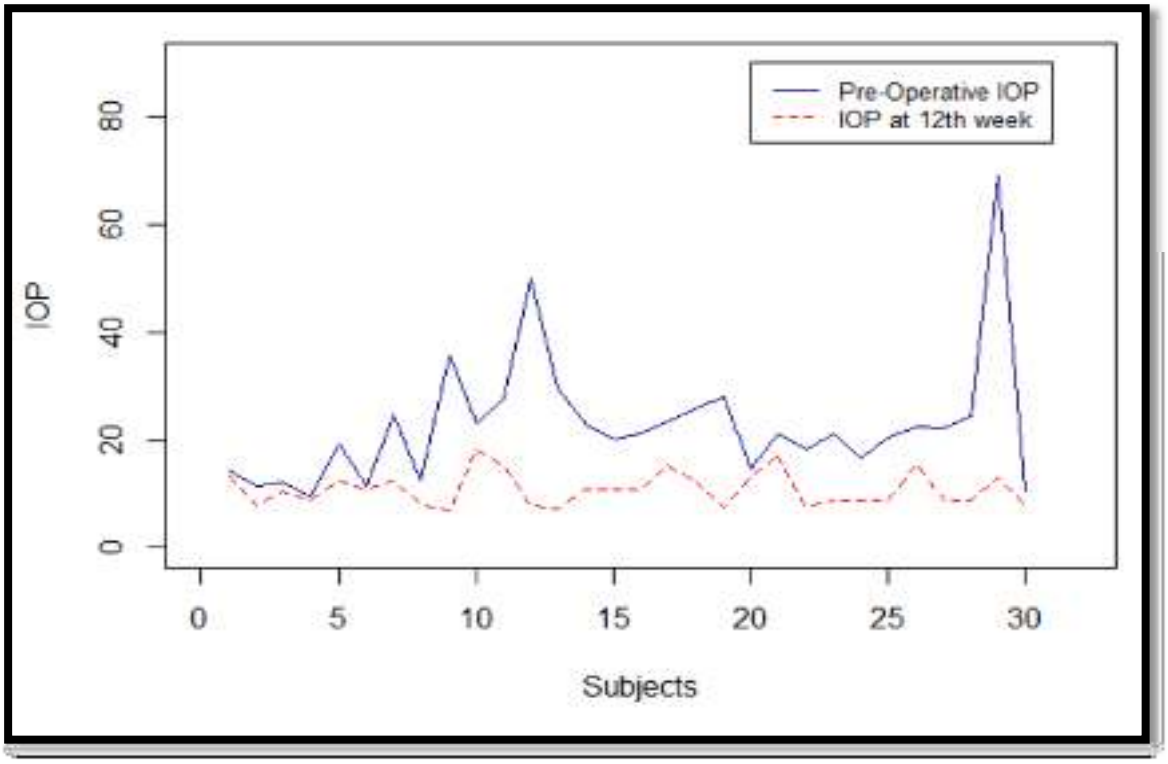


Table 7: Comparison of IOP with type of surgery

Type of Surgery	Time points			P-value
	Pre-Op	Day 1	3rd month	
Trabeculectomy	20.55 ± 5.62	13.05 ± 6.02	11.45 ± 4.77	**< 0.001^{T*}**
Combined Trabeculectomy	23.14 ± 12.78	18.88 ± 7.38	10.85 ± 2.96	0.8361^{G}
				0.1972$^{T:G}$

*Abbreviation: T – Time; G – Group, T*G – Interaction, * indicates statistical significance. Note: Parameters are represented as Mean ± SD.*

In combined trabeculectomy, pre-operative IOP (23.14 ± 12.78mm Hg) is significantly different (P-value < 0.001) from IOP at 3rd month (10.85 ± 2.96mm Hg) and IOP of 1st day (13.05 ± 6.02mm Hg) is significantly different (p-value < 0.001) from IOP at 3rd month.

From repeated measures of ANOVA, we observe that there is no significant interaction effect of time and group. From post hoc analysis, it is observed that, there is no significant difference in IOP over the type of surgery at any time point.

Graph 7: Comparison of IOP with type of surgery

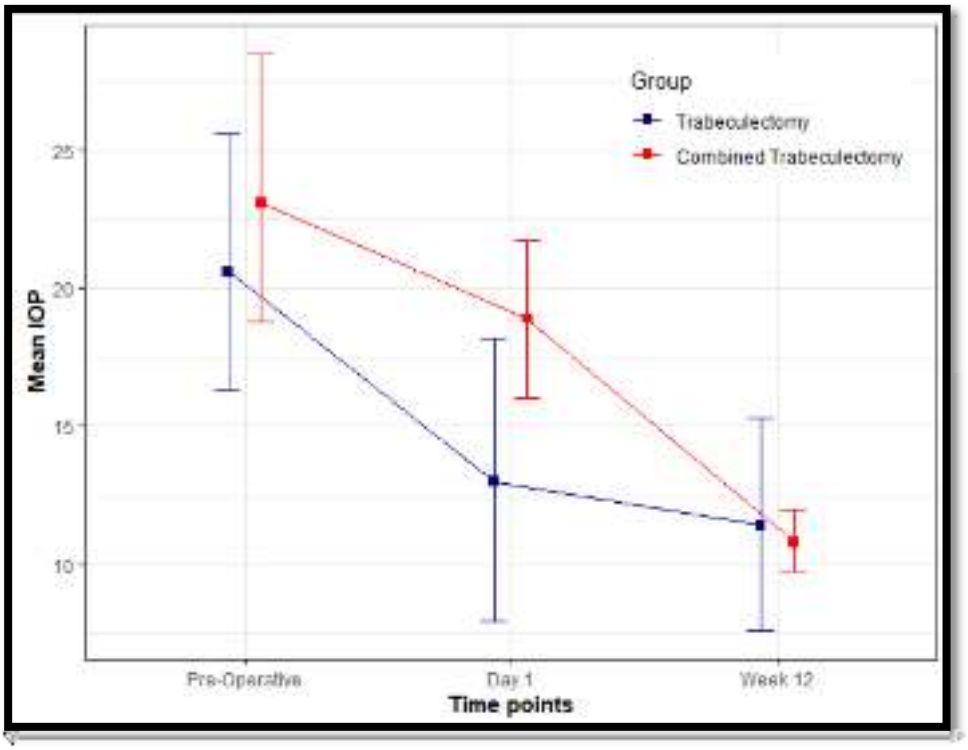


Table 8: Comparison of BCVA before and after the treatment

Preoperative BCVA	Post-Operative BCVA				Total	p-value
	HMCF PL + PR Accurate/ inaccurate-6/60	6/36-6/24	6/18-6/12	6/9-6/6		
HMCF PL+ PR Acc -6/60	5 (100%)	4 (100%)	4 (33.33%)	3 (33.3%)	16	0.0549^{MN}
6/36-6/24	0	0	2 (16.7%)	1 (11.1%)	3	
6/18-6/12	0	0	5 (41.7%)	2 (22.2%)	7	
6/9-6/6	0	0	1 (8.3%)	3 (33.3%)	4	
Total	5	4	12	9	30	

Abbreviation: MN – McNemar - Bowker test.

The post-operative BCVA either remained same or improved. Most of the patients presented with vision ranging from HMCF PL+ PR inaccurate/accurate to 6/60 (16 patients i.e., 53.3%) and post operatively the vision was mostly in the range of 6/18 to 6/12 (12 patients i.e., 40%).From McNemar - Bowker test, we observe that, there is no significant deterioration in the distribution of BCVA before and after surgical intervention.

Graph 8: Comparison of BCVA before and after the treatment

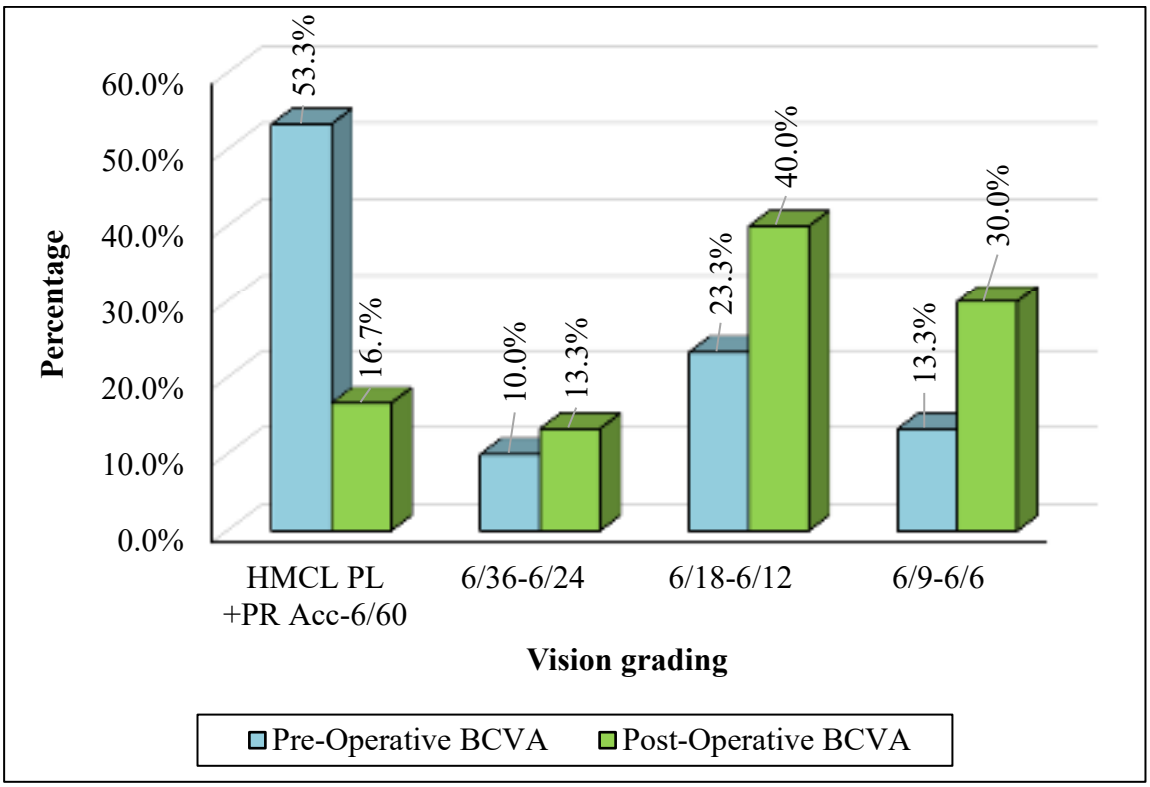


Table 9: Grading of bleb morphologies with time (Moorfield's bleb grading system)

Variables	Sub Category	Time Points		
		Day 1	1st Month	3rd Month
Bleb Height	Flat	1 (3.33%)	1 (3.33%)	2 (6.67%)
	Low	13 (43.33%)	14 (46.67%)	13 (43.33%)
	Moderate	16 (53.33%)	14 (46.67%)	15 (50%)
Bleb Vascularity	Avascular	0	0	2 (6.67%)
	Normal	0	3 (10%)	5 (16.67%)
	Mild	9 (30%)	13 (43.33%)	16 (53.33%)
	Moderate	20 (66.67%)	12 (40%)	7 (23.33%)
	Severe	0	1 (3.33%)	0
Central Bleb area	25%	20 (66.67%)	20 (66.67%)	24 (80%)
	50%	10 (33.33%)	10 (33.33%)	6 (20%)
Peripheral Bleb area	0%	10 (33.33%)	10 (33.33%)	14 (46.67%)
	25%	20 (66.67%)	20 (66.67%)	16 (53.33%)

Maximum number of eyes are found to have a moderately elevated bleb (15 eyes i.e., 50%) and 16 eyes (53.33%) with mild vascularity. 24 eyes (80%) have a central bleb area of 25% and 16 eyes (53.33%) have a bleb area of 25% and a peripheral area of 25% is present in 16 eyes (53.33%) and 0% in 14 eyes (46.67%).

Table 10: Complications of surgery

Complications	No. of patients	Percentage
Hyphaema	1	3.33%
Sub-conjunctival hemorrhage under bleb	1	3.33%
Incomplete Peripheral iridectomy	2	6.67%
Posterior capsule rent	1	3.33%
Toxic anterior segment syndrome	1	3.33%
Nil	24	80%
Buttonholing of conjunctiva	0	0%
Thin – walled blebs	0	0%
Over functioning blebs	0	0%
Bleb hypotony	0	0%
Flat AC with hypotony	0	0%
Hypotonus maculopathy	0	0%
Malignant glaucoma	0	0%
Intraocular infection	0	0%
Sympathetic ophthalmia	0	0%
Dellen	0	0%

It was seen that in 2 patients (6.67%) there was incomplete Peripheral Iridectomy, 1 patient (3.33%) had Sub conjunctival haemorrhage under the bleb, posterior capsule rent occurred in 1 patient (3.33%), 1 patient (3.33%) had hyphaema on post-operative day 1 and 1 patient (3.33%) had toxic anterior segment syndrome.

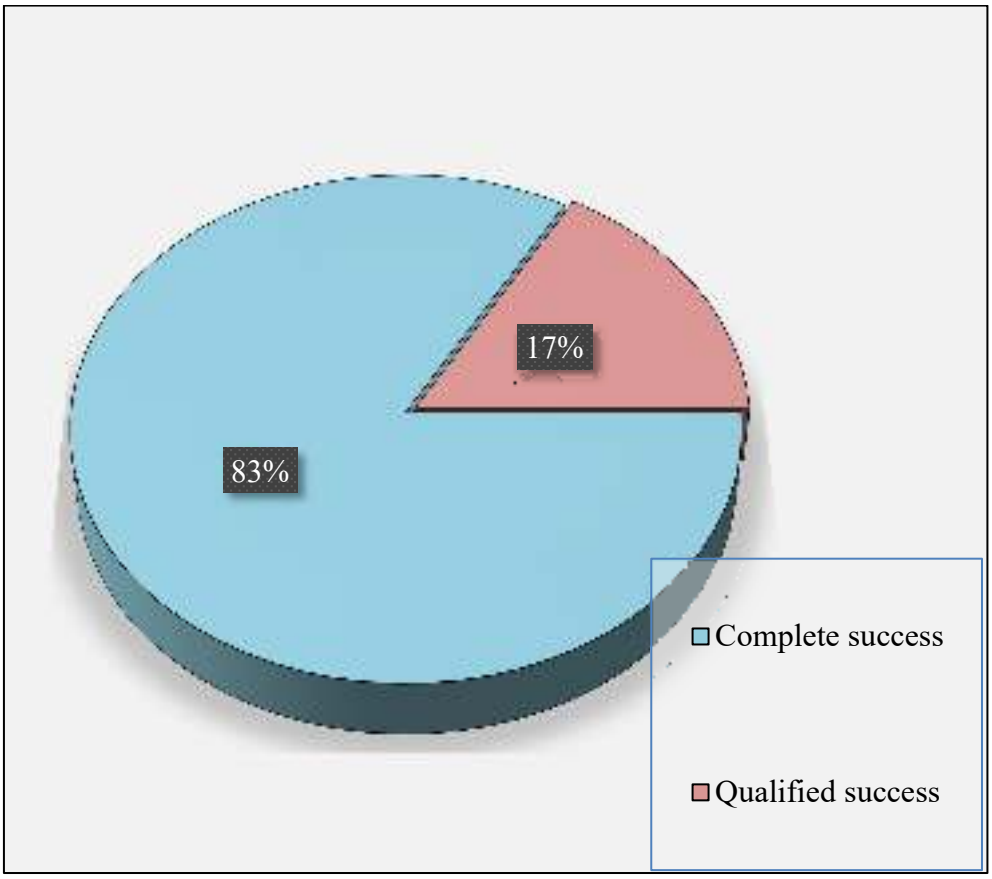
Complications like buttonholing of conjunctiva, thin-walled blebs, over-functioning blebs, flat anterior chamber with hypotony, hypotonous maculopathy, malignant glaucoma, intraocular infection, sympathetic ophthalmia or dellen were not observed in any of the cases in our study.

Table 11: Outcome of surgery

Outcome of surgery	No. of patients	Percentage
Complete success	25	83.33%
Qualified success	5	16.67%
Failed surgery	0	0%
Total success	30	100%

It was observed that majority of the patients, 25 (83.33%) had complete success while the remaining 5 (16.67%) had qualified success. All 30 patients (100%) had total success.

Graph 9: Outcome of surgery



DISCUSSION

The present study was conducted at the Department of Ophthalmology, KLES Dr.Prabhakar Kore Hospital & Medical Research Centre, Belagavi from 1st January 2020 to 31st December 2020 to evaluate the safety and efficacy of amniotic membrane transplantation as an adjunct to trabeculectomy in glaucoma patients. Thirty eyes were enrolled in our study and outcomes were assessed over three months. This is a pioneer study to be conducted in India to our best knowledge.

Age distribution

In our study, majority of the patients were in the range of 61-70 years with a mean age of 65.17 ± 8.23 years. In a study carried out by Eliezer et al[10] in 2006, the control group of 32 patients (without amniotic membrane) showed a mean age of 67.6±8.0 years and the study group of 31 patients (with amniotic membrane) showed a mean age group of 68.3±13.6 years. Another study by Yadava et al in 2017, where they studied 20 eyes in each group revealed similar results with a mean age group of 50.95 ± 9.54 years in one group (using amniotic membrane & Mitomycin-C) and 54.65 ± 11.05 years in the other group (using Mitomycin-C only).[9]

This indicates that increasing age is a considerable risk factor in the development of glaucoma and it correlates with a study that states that population over the age of 60 years were at higher risk.[62]

Gender distribution

In our study, the male to female ratio of 5:1, indicating a higher male predisposition. A study conducted by Stavrakas et al in 2012 showed a male to

female ratio of 1:1 in the control group (non-amniotic) and 19:8 in the study group (amniotic).[12] Eliezer et al had 11 males & 5 females in the group without amniotic membrane and 8 males & 8 females with a male to female ratio of 1:1 in the group using amniotic membrane.

Type of glaucoma

In our study, the distribution was such that 24 patients (80%) were diagnosed with primary open angle glaucoma out of which 50% were in an age range of 61-70 years, followed by 5 (16.67%) with normotensive glaucoma with 2 patients (40%) in both age ranges of 61-70 years and 81-90 years espectively.1 (3.33%) has primary angle closure glaucoma with an age range of 51-60 years.

The study by Yadava et al had a total of 3 patients (7.5%) with POAG and 37 patients (92.5%) with PACG out of a total of 40 patients that were enrolled in the study(9). Drolsum et al conducted a study on patients with refractory glaucoma who underwent trabeculectomy using amniotic membrane out of which 55.5% were patients with POAG and 33.3% were patients with uveitic glaucoma.[57]

The findings are found to be varied in all studies as the number of patients included in these studies is a significantly small number and hence cannot be considered representative of the entire population.

Comorbidities

In our study, out of 30 patients, 13 (54.17%) diagnosed with POAG have no co-morbidities while 7 (29.17%) have hypertension (HTN), 1 (4.17%) has diabetes mellitus (DM) and Ischemic heart disease (IHD) respectively.

Leske et al[63] and Leighton DA[64] et al conducted a study on patients with glaucoma showing that high blood pressure is a significant risk factor for developing POAG. Diabetes mellitus was also found to be a risk factor associated with raised IOP as evidenced by the Rotterdam study[65] and the Baltimore eye survey[66]

IOP reduction

The pre-operative IOP in our study was 22.8 ± 12.04 mmHg. On postoperative day 1 there was a 6.71 ± 52.91 % reduction in the IOP to 18.1 ± 7.4 mm Hg. One week later, the reduction was, 35.24 ± 35.25 % to 12.8 ± 6.01 mm Hg. By 3 months the IOP fell by 43.3 ± 23.65% to a mean of 10.93 ± 3.16 mmHg. The P-value of this is found to be <0.001 which indicates the reduction in IOP is statistically significant.

Other randomized studies evaluating the efficacy of amniotic membrane in glaucoma filtering surgery established similar results. Stavrakas et al showed the median reduction in IOP at 24 months post-surgery was 8 mmHg in the study group (with amniotic membrane) as compared to 6 mmHg in the control group (without amniotic membrane). However the P-value indicated that there was no significant difference in the IOP reduction between the two groups.[12]

Eliezer et al showed a 46% reduction in IOP at 2 months postoperatively and remained steady till 12 months at 13.13±2.50 mmHg in the study group using amniotic membrane which is comparable to our study which showed similar results at the end of 3 months.[10]

A few studies that assessed the efficacy of amniotic membrane as an addition to existing anti-fibrotics such as Mitomycin -C and 5-Fluorouracil. Drolsum et al used two sheets of amniotic membrane impregnated with MMC in patients diagnosed with

refractory glaucoma in which the mean preoperative IOP was 32.2 mmHg .After a mean follow-up of 9.8 months, the mean post-operative IOP was 16.4 mmHg, showing an overall 49% reduction.

Yadava et al conducted a study where glaucoma patients underwent trabeculectomy in which study group was supplemented intraoperatively with both MMC and amniotic membrane transplant (AMT) while control group underwent trabeculectomy with MMC only. In the study group, mean preoperative IOP reduced by 71.09% from 41.9 ± 10.6 mmHg to IOP of 12.1 ± 2.7 mmHg at 1 year, while in control group, a fall of 68.29% was observed from 40.5 ± 8.5 to 12.8 ± 4.5 mmHg.[9] This is at par with the IOP reduction in our study by using amniotic membrane alone.

Preoperative & postoperative Best corrected visual acuity (BCVA)

14 patients (47%) presented with vision ranging from HMCF PL+ PR inaccurate/accurate to 6/60 and post operatively majority of them i.e., 12 patients (40%) the vision was in the range of 6/18 to 6/12 and 9(30%) in the range of 6/9-6/6. We observed no significant deterioration in vision in all the patients. There was either an improvement in the visual acuity (90%) or it remained unchanged (10%) of the patients. The patients whose BCVA remained the same were so due to pre existing glaucomatous damage to the optic nerve head.

Bleb morphology

The result of bleb morphology in our study indicated that majority of the patients had a moderately elevated, mildly vascular bleb with a central bleb area of 25% and a peripheral bleb area of 25%.

Similar results were obtained by Stavrakas et al where the blebs were more diffuse with mild vascularity in the study group (with AMT) and flatter blebs in the control group (without AMT).[12] Eliezer et al showed results that were alike, with 45.16% of the eyes showing elevated and mildly vascular blebs and 56.25% thin avascular blebs in the study group using amniotic membrane while the control group exhibited a majority (62.5%) of flat, vascularized blebs.[10]

This can be attributed to a superior bleb function with a more diffuse area of aqueous drainage and carries a lesser risk of occurrence of thin cystic blebs as seen with the use of antimetabolites. The presence of amniotic membrane in the subconjunctival space may also add to the height of the bleb up until 4 weeks postoperatively beyond which the amniotic membrane dissolves as evidenced by an experimental study conducted by Wang et al.[67]

Complications

The assessment of complications of our study showed us that 2 patients (6.67%) had incomplete peripheral iridectomy, 1 patient (3.33%) had sub conjunctival haemorrhage under the bleb, posterior capsule rent occurred in 1 patient (3.33%), 1 patient (3.33%) had hyphaema on post-operative day 1 and 1 patient (3.33%) had toxic anterior segment syndrome.

Despite incomplete peripheral iridectomy, the 2 patients did not show any significant difference in postoperative IOP or bleb morphology which is comparable to results found by Manners at al.[61] Sub conjunctival hemorrhage and the hyphaema resolved by the 4-week follow up while the TASS was treated with topical and oral steroids. This too did not demonstrate any influence on IOP or bleb characteristics at

the end of 3 months. None of the patients developed any vision threatening complications such as wipe out phenomenon, suprachoroidal haemorrhage, serous choroidal detachment, hypotonus maculopathy, cystoid macular edema, malignant glaucoma, blebitis or endophthalmitis.

Eliezer et al showed complications in only one eye that presented with an encapsulated bleb in the study group (with AMT) and one choroidal detachment and one encapsulated bleb in the control group (without AMT).[10]

However, studies that combined antimetabolites with amniotic membrane such as the one performed by Yadava et al has shown sizeable complications in the control group (that used only MMC) such as choroidal detachment that developed after persistent hypotony in 5%, higher incidence of cataract progression (40%) and 5-FU needling (35%) as opposed to no choroidal detachment and only 15% cases developing cataract and 10% requiring 5-FU needling. This proved that though the results were comparable, the group using amniotic membrane had a lower complication rate and fewer need of interventions. [9]

Outcome

The outcome of surgery was depicted in the form of success rate and it was observed that majority of the patients, 25 (83.33%) had complete success while the remaining 5 (16.67%) had qualified success. All 30 patients (100%) had total success.

As per Eliezer et al 100% patients in the study group (with AMT) used 1-3 antiglaucoma medications pre-operatively which reduced drastically to 18.75% patients using 1 antiglaucoma medication post-operatively resulting in 81.25% having complete success.

The control group (without AMT) had 87.5% of patients using 1-3 antiglaucoma medications which only lowered to 44% using medications post-operatively resulting in only 56% having complete success.[10]

Stavrakas et al again demonstrated similar outcomes, with higher number of complete success in the amniotic membrane group as compared to the non-amniotic membrane group. Although, the difference was not statistically significant.[12]

This suggests a favourable outcome of using amniotic membrane with an agreeable effect on wound healing and bleb characteristics.

CONCLUSION

The present study concludes that trabeculectomy with amniotic membrane transplantation is both safe and efficacious. The reduction in intraocular pressure was clinically significant with a p-value of <0.001, a good bleb morphology of diffuse and mild vascularity by the end of 3 months with all patients having total success. The positive visual outcomes and minimal complications has thus proven that the use of human amniotic membrane as an appendage to conventional trabeculectomy is a novel and effective surgical advancement in the treatment of glaucoma. Hence, amniotic membrane transplantation can be used as an alternative to any of the wound modulators.

SUMMARY

Our study, a prospective, interventional longitudinal study was carried out on 31 eyes who were planned for trabeculectomy to evaluate the safety and efficacy of amniotic membrane transplantation in patients undergoing trabeculectomy.

- In our study, majority of the patients (14 patients i.e., 46.67%) were between 61- 70 years.
- The male to female ratio of 5:1, indicating a higher male predisposition.
- Majority of the patients i.e. 24 (80%) were diagnosed with primary open angle glaucoma, followed by 5 (16.67%) with normotensive glaucoma and 1 (3.33%) with primary angle closure glaucoma.
- Pre-operatively the IOP was 22.8 ± 12.04 mm Hg. On day 1 post-operatively there was a 6.71 ± 52.91 % reduction in the IOP to 18.1 ± 7.4 mm Hg. One week later, the reduction was, 35.24 ± 35.25 % to 12.8 ± 6.01 mm Hg. By 3 months the IOP fell by 43.3 ± 23.65% to a mean of 10.93 ± 3.16 mmHg. The P-value was <0.001 which was statistically significant.
- The BCVA in all patients either remained the same or improved.
- Grading of bleb morphology based on Moorfields bleb grading system showed majority of the cases having a central bleb area of 25% and a peripheral bleb area of 25% which were moderately elevated and mildly vascular blebs.
- Complications observed were that 2 patients (6.67%) had an incomplete peripheral Iridectomy, 1 patient (3.33%) had Sub conjunctival haemorrhage under the bleb ,posterior capsule rent occurred in 1 patient (3.33%), 1 patient

(3.33%) had hyphaema on post-operative day 1 and 1 patient (3.33%) had toxic anterior segment syndrome.

- Complications like thin walled blebs, bleb hypotony, over functioning blebs, suprachoroidal haemorrhage, hypotonous maculopathy, malignant glaucoma, blebitis or endophthalmitis were not observed.
- Surgical outcome as evaluated in the form of success rates of surgery showed 25 patients (83.33%) had complete success while the remaining 5 patients (16.67%) had qualified success. All 30 patients (100%) had total success.
- Therefore, amniotic membrane is a novel and effective adjunct in trabeculectomy.

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ANNEXURE I - INFORMED CONSENT

Title of Research Study: A ONE YEAR PROSPECTIVE STUDY TO EVALUATE THE EFFICACY OF USING AMNIOTIC MEMBRANE AS AN ADJUNCT TO TRABECULECTOMY

PrincipalInvestigator:

DR. _____ M.B.B.S.

POST GRADUATE STUDENT,

DEPARTMENT OF OPHTHALMOLOGY,

JAWAHARLAL NEHRU MEDICAL COLLEGE,

K.L.E. UNIVERSITY, BELAGAVI – 590010

Guide:

DR. _____ M.B.B.S, DOMS, M.S., PhD

PROFESSOR,

DEPARTMENT OF OPHTHALMOLOGY,

JAWAHARLAL NEHRU MEDICAL COLLEGE,

K.L.E. UNIVERSITY, BELAGAVI - 590010

Introduction and Purpose: The purpose of the study is to evaluate the outcomes of using amniotic membrane as an adjunct to trabeculectomy in glaucoma patients at a Tertiary Care Hospital located in Southern India and observe the relative efficacy and individual complications via an observational longitudnal study. Comparisons shall be made primarily between their effect on IOP and bleb formation in a conventional trabeculectomy surgery.

Procedure: If, you agree to be part of the research study, you will be asked the relevant history and will be subjected to relevant clinical examination and investigations.

Risk and Benefits: The risks associated with trabeculectomy surgery are applicable here. You will have good post-operative prognosis and less bleb related complications with this procedure over Conventional Trabeculectomy. Constant monitoring of IOP will be required.

Alternatives: Taking part in this study is voluntary. You may choose not to take part in this study. If you decide to take part you can later change your mind and withdraw from the study. Your decision will not change the present or future health care or other services that you receive. The study doctor or sponsor may stop your participation in this study at any time. If you choose not to take part in the study, you will receive the standard treatment for patients with your condition.

Privacy and Confidentiality: All the information collected about you during the course of this study will be kept confidential to the extent permitted by law. The code numbers will identify you in this research record. Information from this study may be published but your identity will be confidential in any publication.

Institution / Sponsor's policy

Does not apply to this research

Financial incentives for participation

You will not be paid / offered any gifts /incentives for participating in the study.

Authorization to publish the results

The results of the study would be forwarded to the KLE University, Belgaum as part of requirement towards the completion of MS degree, review and publishing.

In case of the queries during study or in future you may contact following

1. Dr. _____ MBBS Investigator, PG in Ophthalmology, J.N.M.C., Belagavi 8095810987	2. Dr.____. MBBS DOMS MS PhD Professor, Dept. of Ophthalmology J.N.M.C., Belagavi 9449938997	3. Dr. _____ MBBS MD DCH Chairman J.N.M.C. Ethical Committee for Human Research J.N.M.C., Belagavi 9448113403

CONSENT FORM: I voluntarily agree to take part in this study by signing below. I may withdraw at any time. I am not giving up any of my legal rights by signing this form. My signature below indicates that I have read this consent form, or it has been read to me, and I have had all the questions answered.

Participant's Name: ……

Name of the Legally Authorized Representative / Guardian: ……

Signature / Left Thumb print of the Participant

or Legally Authorized Representative

Witness' Name: ……

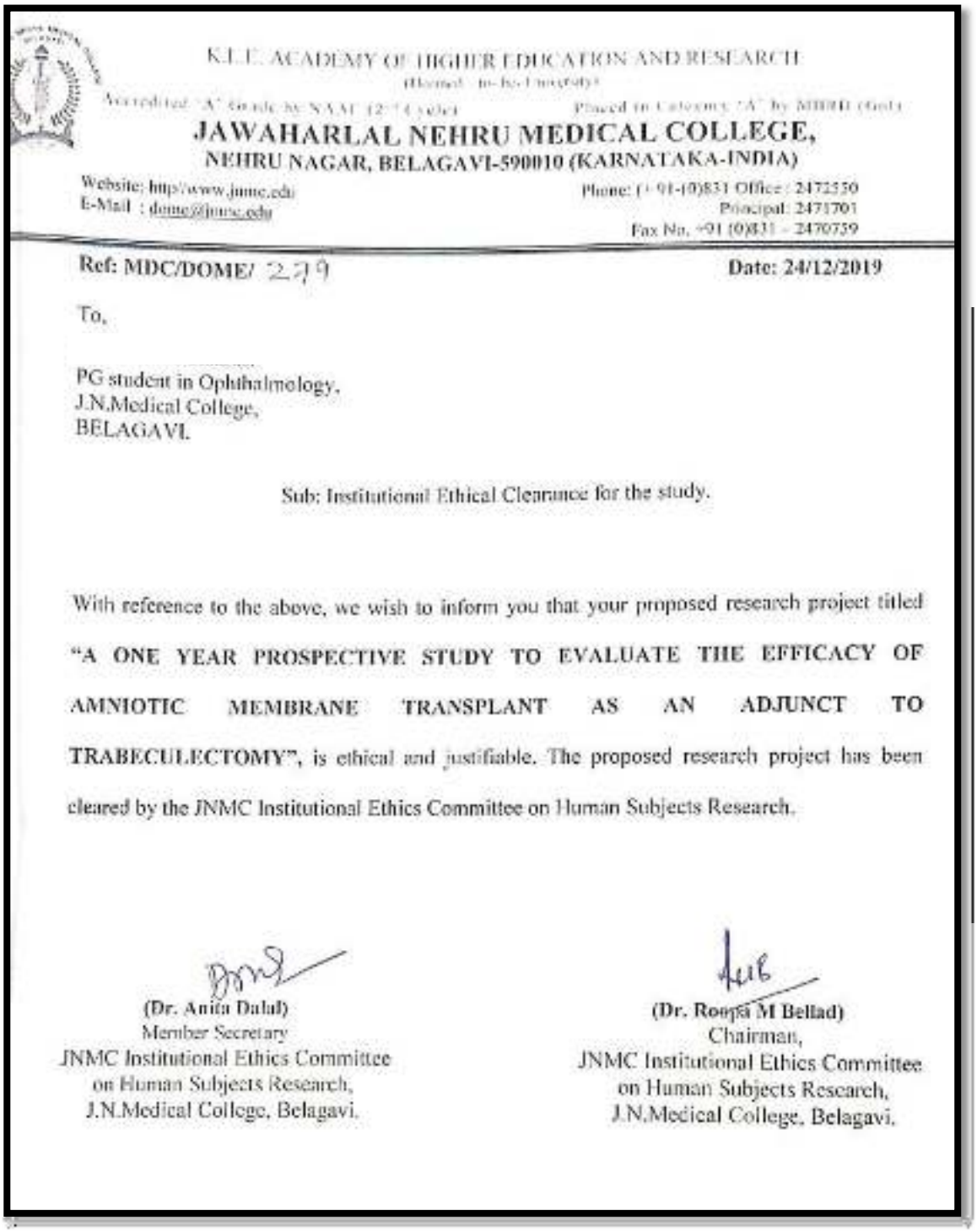
Investigator's Name and Signature: ……

Signature / Left thumb Impression: ……

Signature / Left thumb Impression: ……

DATE: PLACE:

ANNEXURE II – ETHICAL CLEARANCE LETTER



K.L.E. ACADEMY OF HIGHER EDUCATION AND RESEARCH
(Deemed-to-be-University)
Accredited 'A' Grade by NAAC (2nd Cycle) Placed in Category 'A' by MHRD (Govt.)

JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)

Website: http://www.jnmc.edu
E-Mail : dome@jnmc.edu

Phone: (+91-(0)831 Office : 2472550
Principal: 2471701
Fax No. +91 (0)831 - 2470759

Ref: MDC/DOME/ 279 **Date: 24/12/2019**

To,

PG student in Ophthalmology,
J.N.Medical College,
BELAGAVI.

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"A ONE YEAR PROSPECTIVE STUDY TO EVALUATE THE EFFICACY OF AMNIOTIC MEMBRANE TRANSPLANT AS AN ADJUNCT TO TRABECULECTOMY"**, is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

(Dr. Anita Dalal)
Member Secretary
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

(Dr. Roopa M Bellad)
Chairman,
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

ANNEXURE III – PROFORMA

PATIENT INFORMATION:

IP NUMER: OP NUMBER: GLAUCOMA ID:

NAME: _____

AGE:_____ GENDER:F/M PHONE NUMBER:_____

ADDRESS:_____

DATE OF ADMISSION: _____DATE OF DISCHARGE:_____

Is patient eligible for study?

Has informed consent been given?

CHIEF COMPLAINTS:

Diminution of vision: RE/LE/BOTH EYES

Duration:

HISTORY OF PRESENTING ILLNESS:

Diminution of vision: Gradual/Sudden

Progressive/static

Painless/painful

For distance/for near/for both distance and near

Redness: Present/Absent

Watering: Present/Absent

Discharge: Present/Absent

Diplopia: Present/Absent

Coloured halos: Present/Absent

Black spots before the eyes: Present/Absent

Spectacle use: Distance/Near/Both

Duration:_____days/months/years

Last refraction done:_____days/months/years

PAST HISTORY:

Ocular trauma: yes/no

Form of trauma_____

Duration_____days/months/years

Ocular surgery: yes/no

Type of surgery_____

Duration_____days/months/years

Diabetes: yes/no

Duration_____days/months/years

Hypertension: yes/no

Duration_____days/months/year

Any other medical disorders

_____

PERSONAL HISTORY:

Smoking: yes/no

Duration_____days/months/years

Alcoholism: yes/no

Duration_____days/months/years

Other addiction: yes/no

Duration_____days/months/years

GENERAL PHYSICAL EXAMINATION:

Appearance: well-built/moderately built/poorly built/emaciated

Pallor: Present/Absent If present:Mild/Moderate/Severe

Pulse: _____beats/minute BP:_____mm Hg

Temperature:_____°F Respiratory rate:_____/minute

SYSTEMIC EXAMINATION:

CVS: Normal/Abnormal

Specify:_____

RS: Normal/Abnormal

Specify:_____

CNS: Normal/Abnormal

Specify:_____

GIT: Normal/Abnormal

Specify:_____

OCULAR EXAMINATION:

Head posture: Erect/Tilted

Visual axis: Parallel/Deviated

Facial symmetry: Symmetrical/Asymmetrical

Extra-ocular movements: Normal/Restricted/Partially restricted

RE: Binocular: LE:

VISUAL ACUITY:

	RE	LE
DISTANT		
PINHOLE		
NEAR		
WITH GLASSES		

Refraction:-

PRESCRIPTION:

	BCVA	SPHERICAL	CYLINDRICAL	AXIS	SPHERICAL	CYLINDRICAL	AXIS	BCVA	
DVRE									DVLE
NVRE									NVLE

ANTERIOR SEGMENT:

	OD	OS
LIDS		
ADNEXA		
CONJUNCTIVA		
SCLERA		
CORNEA		
ANTERIOR CHAMBER		
IRIS		
PUPIL Size Shape Direct Indirect Near reflex	_____in mm	_____in mm
LENS		

FUNDUS EXAMINATION	OD	OS
GLOW		
MEDIA		
DISC Size Shape Colour NRR Vessels Lamellar dot sign Haemorrhagic spots Other signs		
C:D RATIO		
BLOOD VESSELS		
BACKGROUND		
MACULA		

DIAGNOSIS:

_____

INVESTIGATIONS:

1.Lacrimal Patency

	Patent	Regurgitation	Blocked
		Clear fluid/mucoid flakes	
RE			
LE			

2.IOP

	BY NCT
RE	
LE	

3.Blood Sugar: _____mg% (RBS/FBS)

4. BP:_____mm Hg

5.Gonioscopy:

OD OS

Shaffer's grading-

HIV: R/NR HbsAg: R/NR

BT: _____ min_____ sec

CT: _____ min_____ sec

S. Urea: _____ mmol/L

S. Creat: _____ mg/dL

TREATMENT GIVEN PREOPERATIVELY:

TREATMENT GIVEN PREOPERATIVELY:

Inj. Mannitol 200ml 20% over 20 mins: _____

Tab Diamox 250mg BD: _____

Oflo Eyedrops: _____ Tropicacyl Eyedrops: _____

Pre-Mannitol IOP: OD: mmHg OS: mmHg

Post Mannitol IOP: OD: mmHg OS: mmHg

Pre and Post Mannitol IOP (in mmHg)

	Pre Mannitol IOP			Post Mannitol IOP		
	By Schiotz			By Schiotz		
	5.5g	7.5g	10.0g	5.5g	7.5g	10.0g
RE						
LE						

OPERATIVE PROCEDURE:

Trabeculectomy with Amniotic membrane

Date: _____ Eye to be operated: Right/ Left/Both

ANAESTHESIA: Peribulbar block/ Topical

Pre and Post Block IOP (in mmHg):

	Pre Block IOP			Post Block IOP		
	By Schiotz			By Schiotz		
	5.5g	7.5g	10.0g	5.5g	7.5g	10.0g
RE						
LE						

INCISION: Superior/Temporal/Supero-temporal/Infero-temporal

OPERATIVE COMPLICATIONS: Present/Absent

If present, specify - _____

POST-OPERATIVE COMPLICATIONS: Present/Absent

If present, specify - _____

TREATMENT GIVEN POST OPERATIVELY:

Inj. Mannitol 200ml 20% over 20 mins: _____

Tab Diamox 250mg BD: _____

Ocupol Dx/Gatiquin P Eyedrops:_____ Oflo Eyedrops: _____ Tropicacyl Eyedrops: _____

Pre-Mannitol IOP: OD: mmHg OS: mmHg

Post Mannitol IOP: OD: mmHg OS: mmHg

OPERATING SURGEON: _____

SURGEON'S SIGNATURE:

FOLLOW-UP PLAN: 1DAY / 1WEEK / 1MONTH / 2MONTH / AND 3MONTHS POST - OPERATIVELY

Visual Acuity	RE	LE
DISTANT		
PINHOLE		
NEAR		
AIDED		
Anterior Segment	OD	OS
LID		
ADNEXA		
CONJUNCTIVA		
SCLERA		
CORNEA		
ANTERIOR CHAMBER		
IRIS		
PUPIL		
LENS		

Fundus Examination	OD	OS
GLOW		
MEDIA		
DISC		
C:D RATIO		
BLOOD VESSELS		
BACKGROUND		
MACULA		

IOP (IN mm Hg)	BY NCT
RE	
LE	

OUTCOME: Improved/Deteriorated

Bleb Morphology: Size: _____ Flat/Diffuse Vascular/Avascular Sutures

+/- Moorfield's Bleb Grading:

Diffusion area: Central – 0% 25% 50% 75% 100%

Peripheral – 0% 25% 50% 75% 100%

Bleb Height: Flat/Low/Moderately Elevated/Max Elevated

Vascularity: Acystic/Avascular/Normal/Mild/Moderate/Severe

Treatment Prescribed:_____

COMPLICATIONS:

Buttonholing of conjunctiva	Hyphema	Thin – walled blebs
Flat AC with hypotony	Intraocular Infection	Overfunctioning Blebs
Malignant glaucoma	Sympathetic Ophthalmia	Bleb Hypotony
Suprachoroidal haemorrhage	Hypotonous Maculopathy	Dellen

FOLLOW-UP PLAN: 1 WEEK POST-OPERATIVELY

Visual Acuity	RE		LE	
DISTANT				
PINHOLE				
NEAR				
AIDED				

Anterior Segment	OD	OS
LID		
ADNEXA		
CONJUNCTIVA		
SCLERA		
CORNEA		
ANTERIOR		

CHAMBER		
IRIS		
PUPIL		
LENS		

Fundus Examination	OD	OS
GLOW		
MEDIA		
DISC		
C:D RATIO		
BLOOD VESSELS		
BACKGROUND		
MACULA		

IOP (IN mm Hg)	BY NCT
RE	
LE	

OUTCOME: Improved/Deteriorated

Bleb Morphology:

Size: _____ Flat/Diffuse Vascular/Avascular Sutures +/-Moorfield's

Bleb Grading:

Diffusion area: Central – 0% 25% 50% 75% 100%

Peripheral – 0% 25% 50% 75% 100%

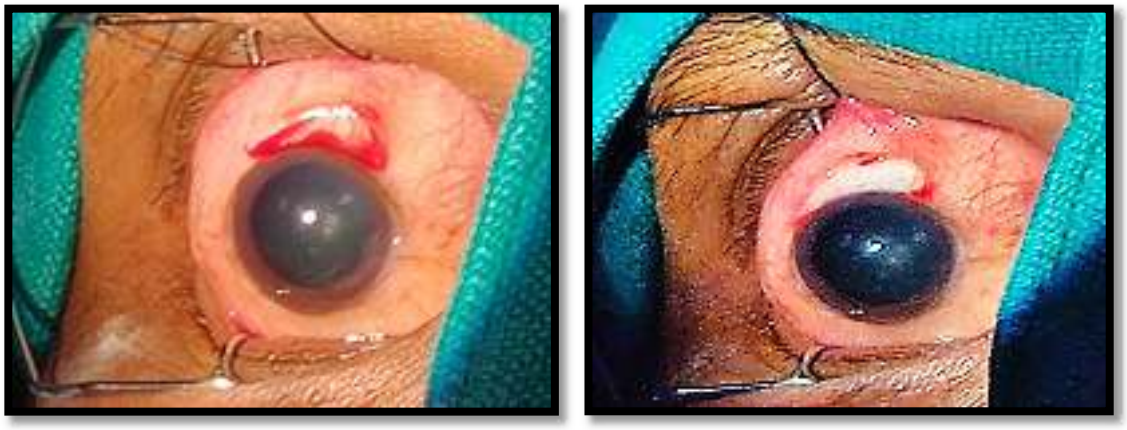
Bleb Height: Flat/Low/Moderately Elevated/Max Elevated

Vascularity: Acystic/Avascular/Normal/Mild/Moderate/Severe

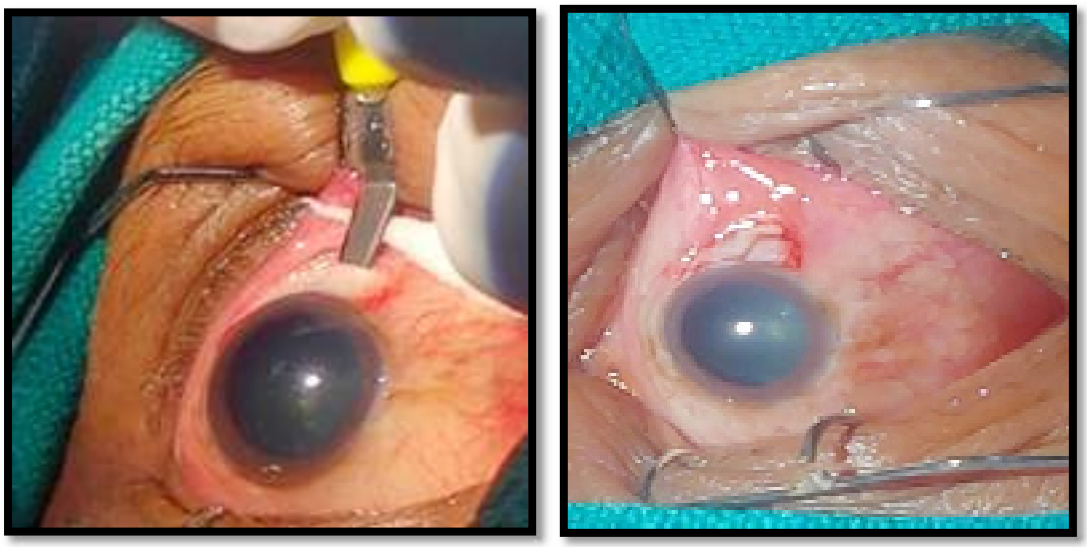
Treatment Prescribed:_____

ANNEXURE IV – PHOTOGRAPHS

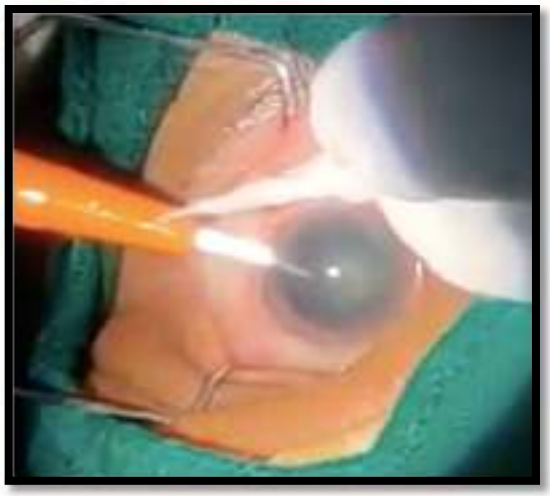
Picture 1: Fornix based conjunctival flap raised



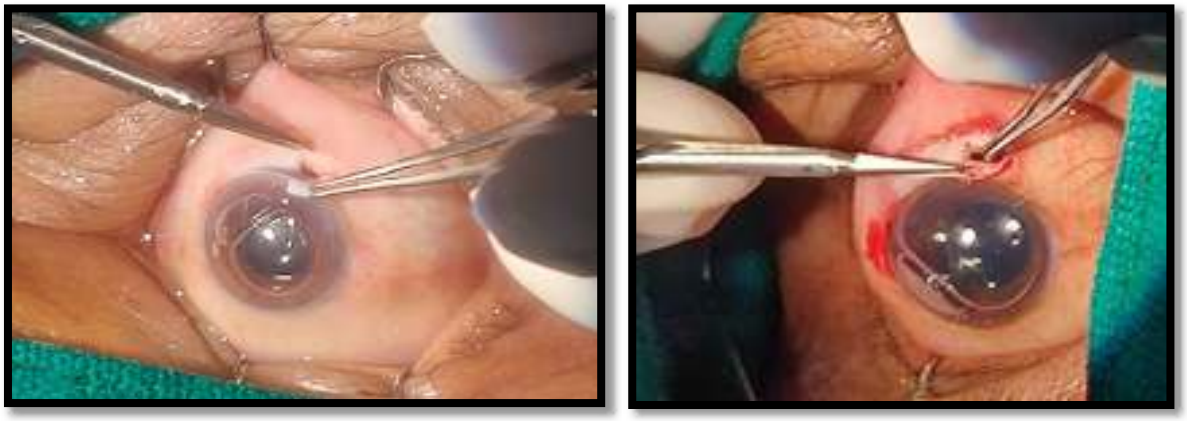
Picture 2: Rectangular scleralflap being raised



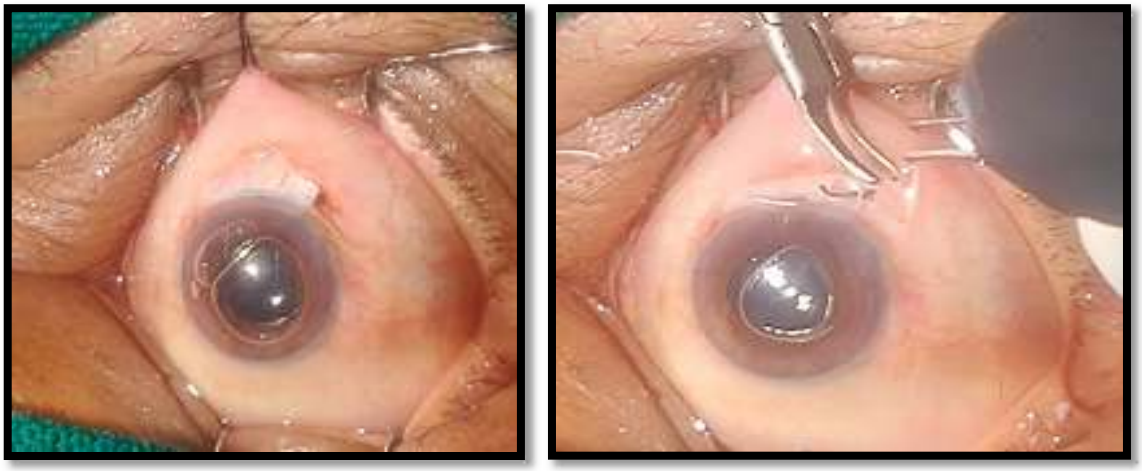
Picture 3: Paracentesis being performed



Picture 4: Sclerostomy and peripheral iridectomy

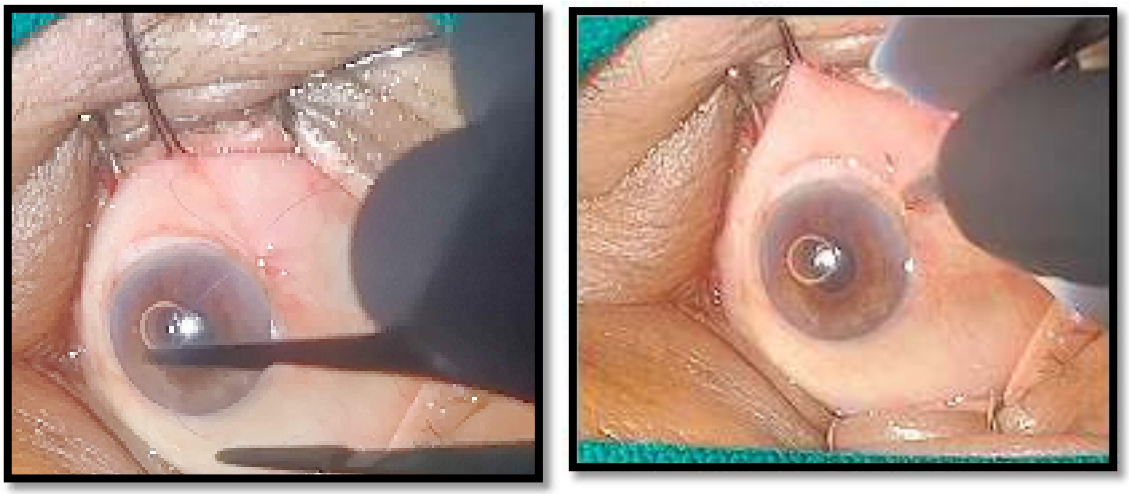


Picture 5: Scleral flap being sutured

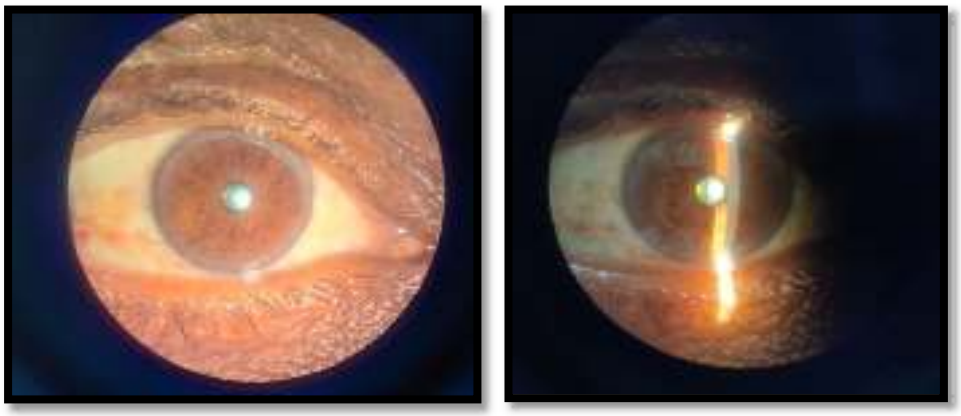


Picture 6: Amniotic membrane transplantation

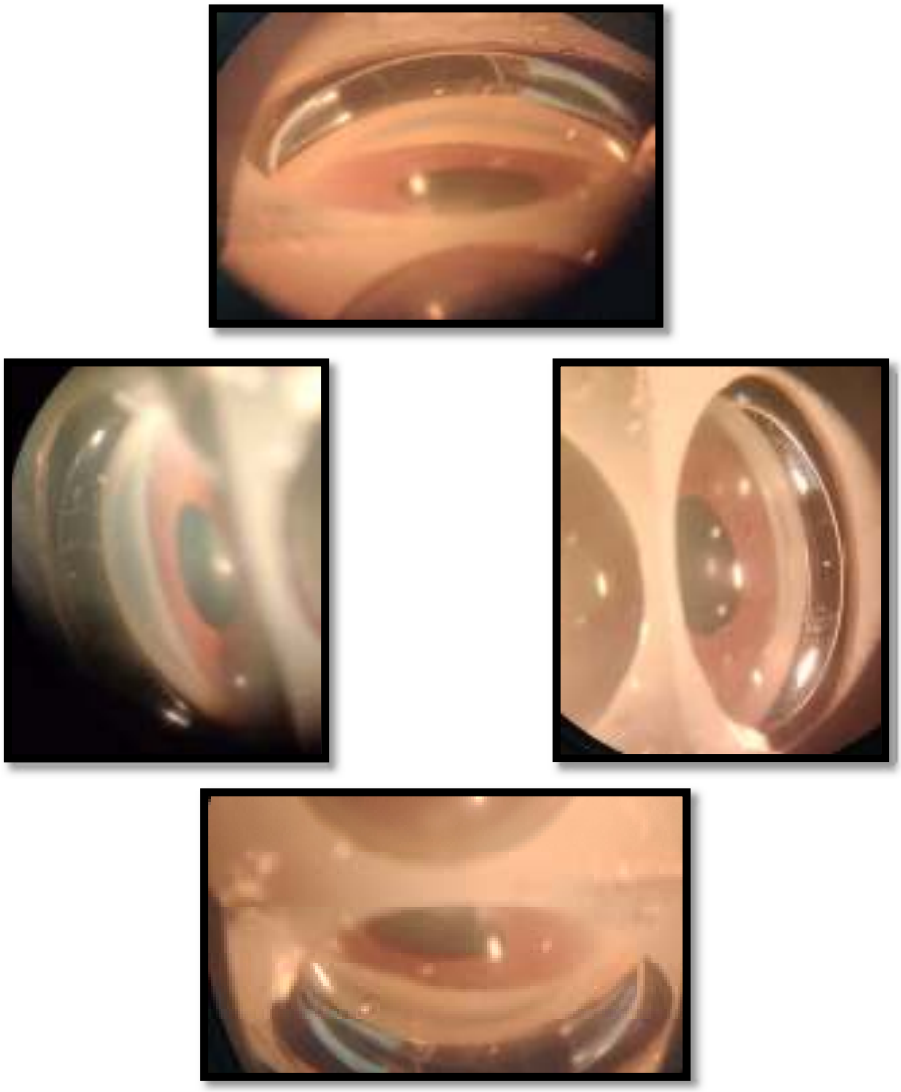
Picture 7: Closure of conjunctival flap



Picture 8: Pre-operative Evaluation (Slit lamp examination)

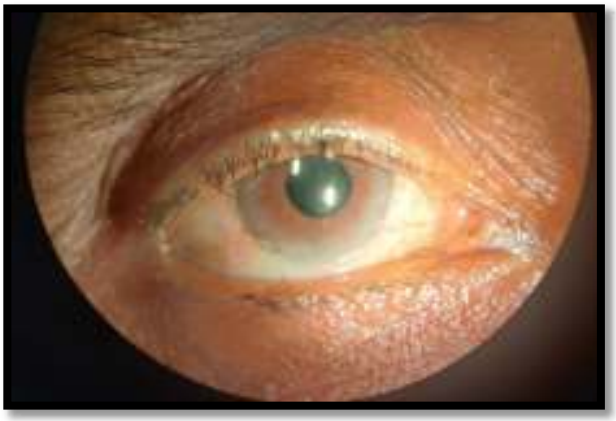


Picture 9: Gonioscopy using a Goldmann 3-mirror lens

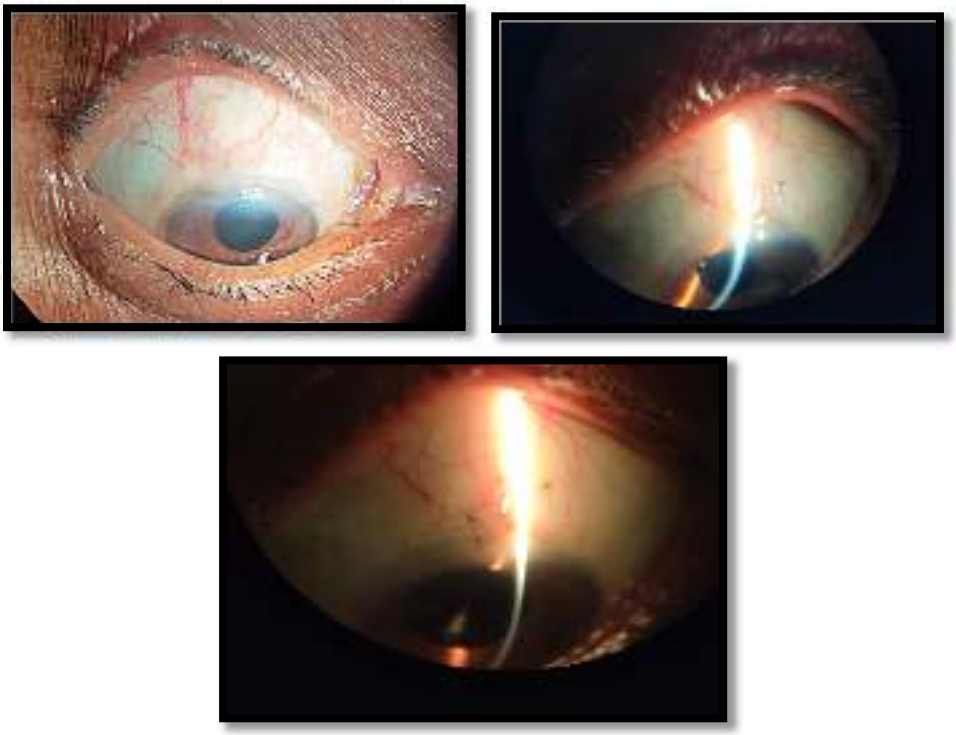


Picture 10: Visual field examination

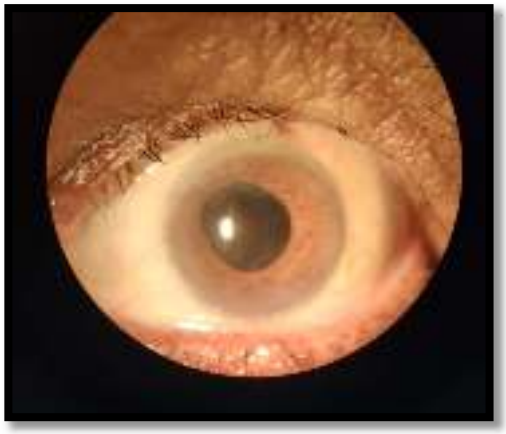
Picture 11:Post-operative Day 1 Slit lamp examination



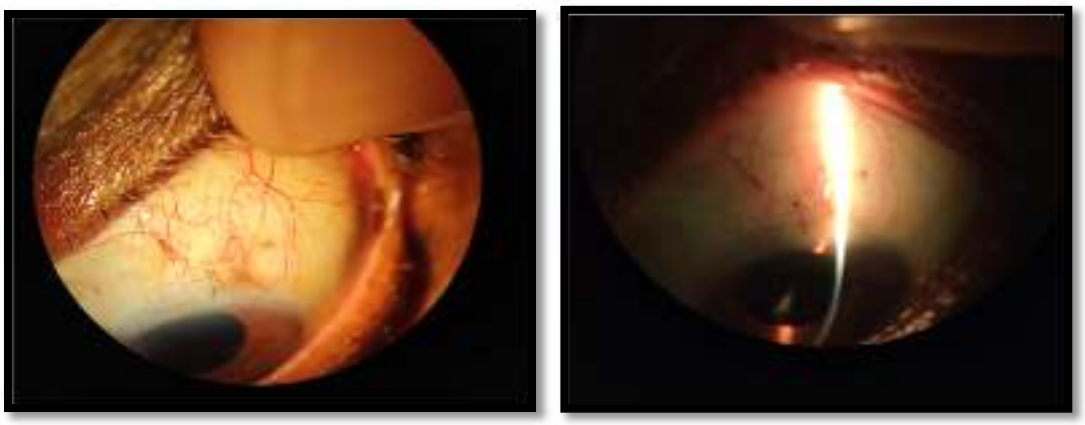
Picture 12: Post-operative Day 1 Bleb morphology



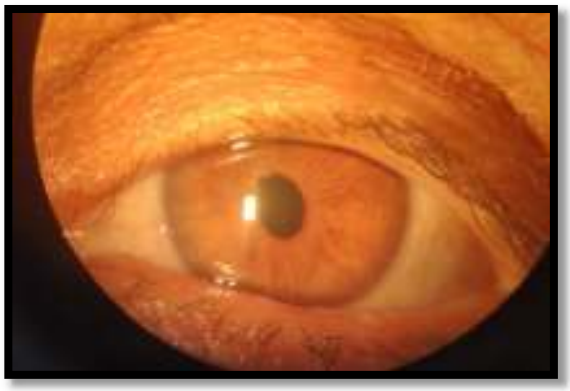
Picture 13: Post-operative 1 week Slit lamp examination



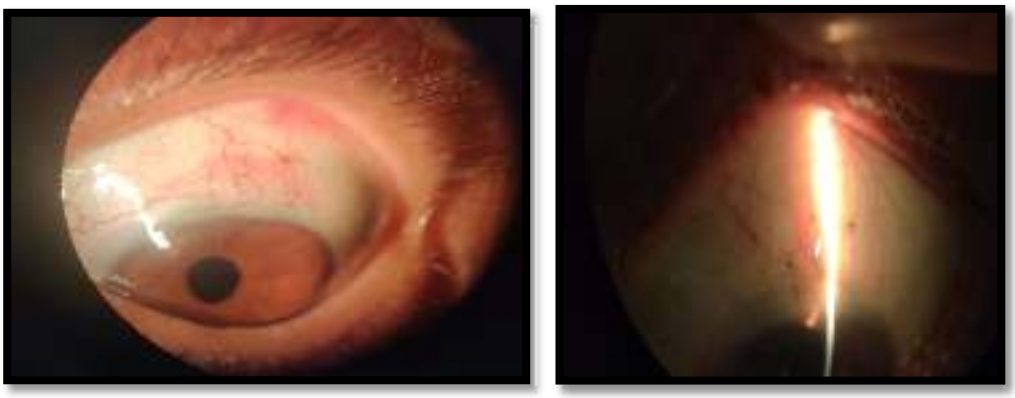
Picture 14: Post-operative 1 week bleb morphology



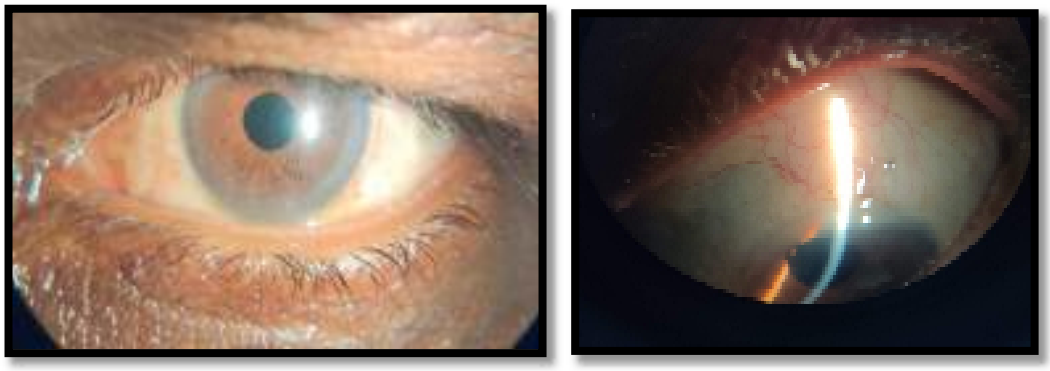
Picture 15: Post-operative 1 month slit lamp examination



Picture 16: Post-operative 1 month bleb morphology

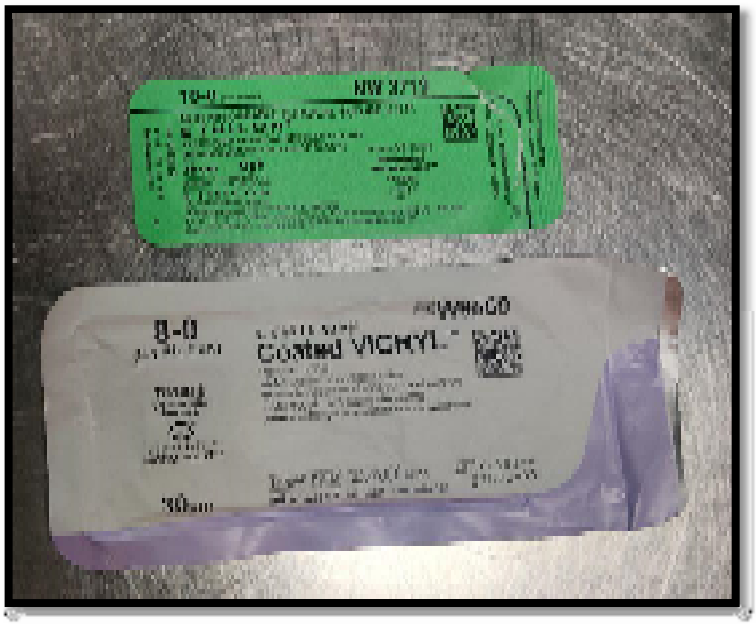


Picture 17: Post-operative 3 months slit lamp examination and bleb morphology



Picture 18: Trabeculectomy

Picture 19: Suture materials



ANNEXURE V– KEY TO MASTER CHART

IOP	:	Intraocular pressure
BCVA	:	Best Corrected Visual Acuity
CF	:	Counting Fingers
HMCF	:	Hand Movement Close to Face
PL+ PR	:	Perception Of Light, Projection Of Rays
M	:	Male
F	:	Female
POAG	:	Primary open angle glaucoma
PACG	:	Primary angle closure glaucoma
NTG	:	Normotensive Glaucoma
R	:	Right
L	:	Left
Mt	:	Metres
CS	:	Complete Success
QS	:	Qualified Success
COMBINED	:	Combined surgery
TRAB	:	Trabeculectomy
SCH	:	Subconjunctival Haemorrhage
PI	:	Peripheral Iridectomy

ANNEXURE VI– MASTER CHART

SI.NO	AGE/SEX	CO-MORBIDITIES	EYE INVOLVED	PRE-OP BCVA	PRE-OP IOP	DIAGNOSIS	TYPE OF SURGERY	DAY 1		WEEK 1		WEEK 2		WEEK 4 (1 month)		WEEK 8 (2 months)		WEEK 12 (3months)		Percentage reduction in IOP	BLEB CHARACTERISTICS				OUTCOME	COMPLICATIONS
								POD 1 VA	POD1 IOP	POD WEEK 1 VA	POD WEEK 1 IOP	POD WEEK 2 VA	POD WEEK 2 IOP	POD 1st MONTH VA	POD 1st MONTH IOP	POD 2nd MONTH VA	POD 2nd MONTH IOP	POD 3rd MONTH VA	POD 3rd MONTH IOP		BLEB HEIGHT	BLEB VASCULARITY	CENTRAL BLEB AREA	PERIPHERAL BLEB AREA		
PATIENT 1	82/M	NIL	L	CF 1/2 Mt	14.4	NTG	COMBINED	6/36	35	6/18	8.6	6/24	13.5	6/6(P)	11.9	6/6(P)	16.9	6/9(P)	13.4	6.9	Moderately elevated	moderate	25%	0%	CS	
PATIENT 2	80/M	NIL	L	HMCF PL+ PR ACC	11.5	NTG	COMBINED	PL+ PR ACC	8.5	PL+ PR ACC	7.2	PL+ PR ACC	7.3	PL+ PR ACC	7.5	PL+ PR ACC	8.6	PL+ PR ACC	8	30.4	Moderately elevated	mild	25%	25%	CS	
PATIENT 3	66/F	HTN	R	CF 1/2 Mt	13.8	POAG	COMBINED	6/24	23.1	6/18(P)	14.3	6/60	9.4	6/18	7.8	6/18	13.8	6/18	10.4	14.75	low	moderate	25%	0%	CS	
PATIENT 4	72/M	HTN	L	6/9(P)	9.5	NTG	COMBINED	6/18(P)	18.9	6/18(P)	6.6	6/18	10.2	6/12(P)	12.2	6/12(P)	9	6/12(P)	9	5	low	mild	25%	25%	CS	SCH UNDER BLEB
PATIENT 5	75/M	DM HTN	L	6/18	19.1	POAG	COMBINED	PL+ PR ACC	10.1	PL+ PR ACC	34	PL+ PR ACC	32	PL+ PR ACC	22.1	6/60	24.3	6/18(P)	12.6	34.03	Moderately elevated	mild	25%	25%	QS	
PATIENT 6	62/F	NIL	R	6/24	26.2	POAG	COMBINED	6/9	11.4	LOST FOR FOLLOW UP DUE TO LOCKDOWN(COVID-19)																
PATIENT 7	65/F	NIL	R	CF 3Mt	11.5	NTG	COMBINED	6/9(P)	18.3	LOST FOR FOLLOW UP DUE TO LOCKDOWN(COVID-19)								6/18	10.9		Moderately elevated	mild	25%	25%	CS	
PATIENT 8	75/F	HTN	L	6/60	24.6	POAG	COMBINED	6/60	23.9	6/60	11.7	6/60	31.5	6/60	13.9	6/60	14.4	6/60	12.6	48.8	Moderately elevated	mild	25%	25%	CS	
PATIENT 9	69/F	DM	R	HMCF PL+ PR ACC	12.4	NTG	COMBINED	6/36(P)	12.7	6/18	7.2	6/12	8.9	6/12	8.1	6/12	9.2	6/12	8.2	33.9	Moderately elevated	mild	25%	25%	CS	
PATIENT 10	68/M	NIL	R	HMCF PL+ PR ACC	35.9	POAG	COMBINED	HMCF PL+ PR ACC	19.5	HMCF PL+ PR ACC	7.2	HMCF PL+ PR ACC	10	HMCF PL+ PR ACC	20.9	HMCF PL+ PR ACC	7.6	HMCF PL+ PR ACC	7	80.5	low	mild	25%	0%	QS	TASS
PATIENT 11	57/M	HTN	R	6/18	23.1	POAG	COMBINED	6/12	30					6/12	11.5	6/12	17.3	6/18	18.2	21.2	low	mild	50%	0%	CS	
PATIENT 12	66/M	DM	R	CF 1/2 Mt	27.5	POAG	COMBINED	CF CF	20.1	CF CF	17.2	6/36	10.4	6/24	14.2	6/24	14.2	6/24	15.4	44	low	moderate	50%	25%	CS	
PATIENT 13	55/M	IHD, HTN	L	6/36	50	POAG	COMBINED	6/12	20.4	6/18	22.3	6/12	44.6	6/12	13.2	6/12	27.8	6/12	7.9	84.2	low	moderate	50%	25%	QS	INCOMPLETE PI
PATIENT 14	68/M	NIL	L	6/24	29.6	POAG	COMBINED	6/18	24.7	6/18	10.9	6/18	22.5	6/18	6.8	6/18	7	6/18	7.2	75.7	moderately elevated	moderate	25%	25%	QS	
PATIENT 15	66/M	NIL	L	6/36(P)	22.9	POAG	COMBINED	6/9	21.9	6/9	6.5	6/9	15.7	6/9	18.6	6/9	12.4	6/9	11.4	50.2	low	moderate	25%	0%	CS	
PATIENT 16	57/M	HTN	L	6/24	20.2	POAG	COMBINED	HMCF PL+ PR ACC	30	6/18	24.4	6/18	6.5	6/18	16.5	6/24	16.4	6/24	14.4	28.7	low	mild	25%	25%	CS	PC RENT, RIGID PCIOL IN SULCUS
PATIENT 17	55/M	HTN,IHD	R	6/18	21.2	POAG	COMBINED	6/24	22	6/18	15.3	6/18	33.5	6/12	8.7	6/12	11	6/12	14	34	low	mild	25%	0%	QS	
PATIENT 18	53/M	NIL	R	6/12	23.3	POAG	COMBINED	6/36	9.5	6/12	10.7	6/12	24.1	6/6	15.3	6/6	14.4	6/6	15.2	34.6	low	mild	50%	25%	CS	INCOMPLETE PI
PATIENT 19	61/M	IHD	L	HMCF PL+ PR ACC	26	POAG	COMBINED	PL+ PR Inacc	27.5	6/36	10.5	6/36	13.4	6/36	14.8	6/36	11.6	6/36	10.5	61.8	moderately elevated	moderate	25%	0%	CS	GRADE 4 HYPHAEMA
PATIENT 20	64/M	NIL	R	6/6	28	POAG	TRAB	6/6	8.8	6/6	9	6/6	7.9	6/6	8.4	6/6	7.7	6/6	7.5	80	moderately elevated	normal	50%	25%	CS	

SI.NO	AGE/SEX	CO-MORBIDITIES	EYE INVOLVED	PRE-OP BCVA	PRE-OP IOP	DIAGNOSIS	TYPE OF SURGERY	DAY 1		WEEK 1		WEEK 2		WEEK 4 (1 month)		WEEK 8 (2 months)		WEEK 12 (3months)		Percentage reduction in IOP	BLEB CHARACTERISTICS				OUTCOME	COMPLICATIONS
								POD 1 VA	POD1 IOP	POD WEEK 1 VA	POD WEEK 1 IOP	POD WEEK 2 VA	POD WEEK 2 IOP	POD 1st MONTH VA	POD 1st MONTH IOP	POD 2nd MONTH VA	POD 2nd MONTH IOP	POD 3rd MONTH VA	POD 3rd MONTH IOP		BLEB HEIGHT	BLEB VASCULARITY	CENTRAL BLEB AREA	PERIPHERAL BLEB AREA		
PATIENT 21	67/M	NIL	L	6/9	22.2	POAG	TRAB	6/9	16.9	6/9	17.5	6/9	16.2	6/9	14.6	6/9	17.4	6/9	13.2	40.5	moderately elevated	mild	50%	25%	CS	
PATIENT 22	58/M	NIL	R	6/18	21.1	POAG	TRAB	6/18	19.4	6/18	15	6/18	18.4	6/18	16.2	6/18	15.2	6/18	17.4	18	low	mild	25%	0%	CS	
PATIENT 23	79/M	NIL	R	6/12	18.4	POAG	TRAB	6/12	7.1	6/12	6.9	6/12	7.4	6/12	7	6/12	8.2	6/12	7.7	58	moderately elevated	NORMAL	25%	0%	CS	
PATIENT 24	65/M	NIL	R	6/18	21.2	POAG	COMBINED	6/18	15.9	6/12	15.9	6/9	9.4	6/9	10.5	6/9	9.2	6/9	8.9	58	moderately elevated	MILD	25%	0%	CS	
PATIENT 25	63/M	NIL	L	6/18	16.5	POAG	COMBINED	6/18	20.4	6/9	7.8	6/9	8	6/9	7.7	6/9	8.4	6/9	8.9	46	moderately elevated	mild	25%	0%	CS	
PATIENT 26	70/M	HTN	L	PL+ PR Acc	20.6	POAG	COMBINED	PL + PR Acc	16.1	PL + PR Acc	13.4	PL + PR Acc	10.8	PL + PR Acc	7.2	PL + PR Acc	9.1	PL + PR Acc	8.8	58	low	avascular	25%	25%	CS	
PATIENT 27	60/M	NIL	L	CF 3Mt	22.4	POAG	COMBINED	6/12	13.1	6/9	12.8	6/9	13.1	6/9	14.4	6/9	17.9	6/9	15.6	29	moderately elevated	normal	25%	0%	CS	
PATIENT 28	50/M	NIL	L	CF 2Mt	22.2	POAG	COMBINED	CF 4M	10.2	CF 5M	12.4	CF 5M	7.2	CF 5M	6.7	CF 5M	7.8	CF 5M	8.9	60	moderately elevated	mild	25%	0%	CS	
PATIENT 29	65/M	NIL	L	CF 1Mt PL+ PR ACC	24.4	POAG	COMBINED	6/60	7.9	6/24	8.4	6/24	5.2	6/24	9.8	6/24	9.5	6/24	8.9	64	flat	mild	25%	25%	CS	
PATIENT 30	54/M	DM	R	6/18	69.1	PACG	COMBINED	6/36	22.6	6/18	14.6	6/18	10.6	6/18	11.9	6/18	12	6/18	13.2	80	low	normal	25%	0%	CS	
PATIENT 31	70/F	HTN	L	6/18	10.4	POAG	COMBINED	6/36	8.6	6/36	10	6/36	9.4	6/36	11	6/36	7.6	6/36	7.9	24	low	mild	25%	25%	CS	