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CLINICAL STUDY ON EFFECT OF SUBCONJUNCTIVAL INJECTION OF BEVACIZUMAB ON CORNEAL NEOVASCULARIZATION

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CERTIFICATE

This is to certify that the dissertation entitled, "**CLINICAL STUDY ON EFFECT OF SUBCONJUNCTIVAL INJECTION OF BEVACIZUMAB ON CORNEAL NEOVASCULARIZATION"** submitted by **Dr. R. NIRAIMOZHI,** in partial fulfillment for the award of the degree of Master of Surgery in Ophthalmology by the Tamilnadu Dr. M.G.R Medical University, Chennai is a bonafide record of work done by her in the Regional Institute of Ophthalmology, Government Ophthalmic Hospital, Egmore, Chennai, during the academic year 2008 – 2011.

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CLINICAL STUDY ON EFFECT OF SUBCONJUNCTIVAL INJECTION OF BEVACIZUMAB ON CORNEAL NEOVASCULARIZATION

PART I

INTRODUCTION

The cornea has the unique feature of being normally avascular except for small loops which invade the periphery for about 1mm. But under pathologic conditions vessels invade the cornea from the limbal vascular plexus. A wide variety of insults including

- infection,
- inflammation,
- ischemia,
- degeneration,
- trauma, and
- loss of the limbal stem cell barrier can cause corneal

neovascularization. Although corneal NV can occasionally serve a beneficial role in the clearing of infections, wound healing, and in arresting stromal melts, its disadvantages are numerous. Corneal NV often leads to tissue scarring, Oedema, lipid deposition, and persistent inflammation that may significantly alter visual acuity. Corneal NV accompanies the most common causes of corneal infectious blindness in both the developed (herpetic keratitis) and developing (trachoma and onchocerciasis) world, which cause millions to lose their sight.

Neovascularization is divided into two main processes; Vasculogenesis and Angiogenesis. The former Vasculogenesis is the formation of new blood vessels from bone marrow derived angioblast, while the latter is formation of new blood vessels from already present vascular structures.[1]

Corneal NV may not only reduce visual acuity but also it results in the loss of the immune privilege of the cornea, thereby worsening the prognosis of subsequent penetrating keratoplasty (PK). Pre-existing corneal stromal blood vessels have been identified as strong risk factor for immune rejection after corneal transplantation. Although various compounds have been shown to inhibit corneal neovascularization in experimental and clinical situations including steroids, indomethacin, cyclosporine, methotrexate, low molecular weight heparin sulphate, rapamycin and thalidomide there is still no clear consensus about the best treatment of corneal neovascularization. Although steroids are the mainstay of treatment in corneal neovascularization, they are not always effective and without complications[1].

FUNCTIONAL ANATOMY & FINE STRUCTURE OF CORNEA

The cornea is a transparent, avascular, watch glass like structure. It forms anterior one sixth of the outer fibrous coat of the eyeball. Has five layers Epithelium, Bowman's membrane, Stroma, Descemet's membrane, and Endothelium.

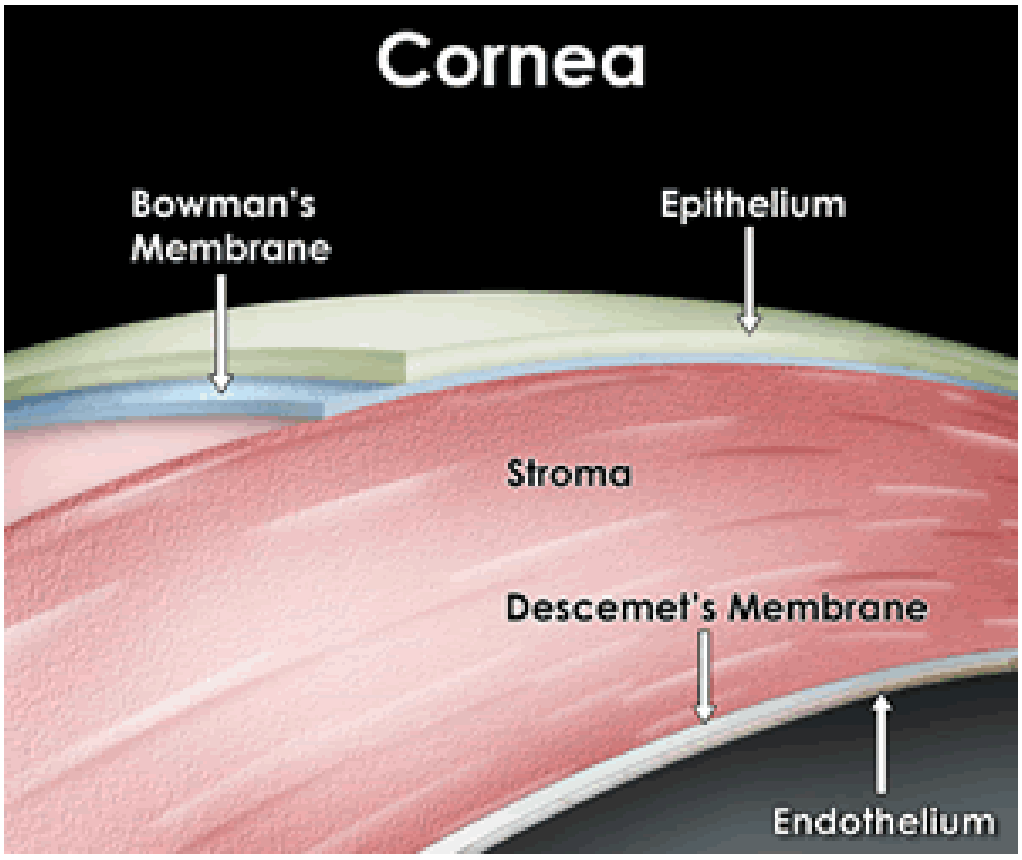


Fig. 1 Layers Of the cornea

EPITHELIUM

Corneal epithelium is of stratified squamous type and becomes continuous with epithelium of bulbar conjunctiva at the limbus. It is about 50 – 90 micrometer thickness and consists of 5 – 6 layers of cells. The deepest basal layer is made up of columnar cells, next 2 – 3 layers of wing or umbrella cells and the more superficial 2 layers are of flattened cells. The corneal epithelium sheds at irregular intervals and is replaced by growth from its basal cells. It is estimated that the entire epithelium is replaced in a period of 6 – 8 days.

BOWMAN'S MEMBRANE

This layer consists of acellular mass of condensed collagen fibrils. It is about 8 – 14 micrometer in thickness and binds the corneal stroma anteriorly with basement membrane of the epithelium. It is not a true elastic membrane but simply a condensed superficial part of the stroma. It shows considerable resistance to infection and injury. But once destroyed does not regenerate.

STROMA (SUBSTANTIA PROPRIA)

This layer is about 0.5mm in thickness and constitutes most of the cornea (90% of total thickness). It consists of collagen fibrils (lamellae) and cells embedded in hydrated matrix of proteoglycans.

The lamellae are arranged in many layers, in each layer they are not only parallel to each other but also to the corneal plane and become continuous with the scleral lamella at the limbus. They vary in disposition according to the area of the cornea. They have oblique orientation in the anterior 1/3rd of the stroma. In the posterior 2/3rd of the stroma the alternating layer of lamella are at right angles to each other.

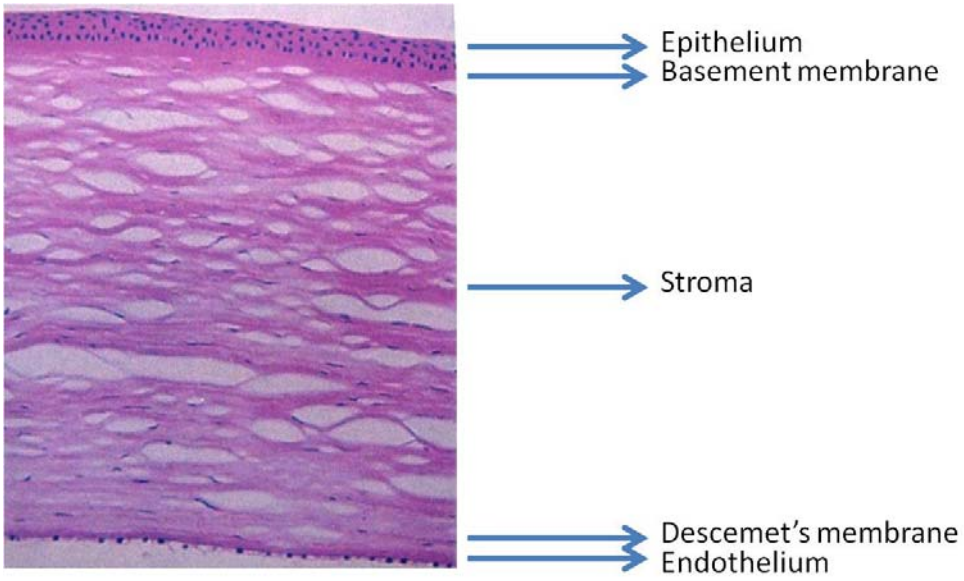


Fig 2. Histology of cornea

The parallel arrangement of lamella in the cornea allows an easy intralamellar dissection during superficial keratectomy and lamellar keratoplasty. The peculiar arrangement of lamellae has also been implicated in the corneal transparency.

The cells among the lamellae are present keratocytes, wandering macrophages, histiocytes and few lymphocytes. The corneal keratocytes constitute 2 – 4 % of the volume of the stroma in humans. The keratocytes are fibroblasts which are found throughout the stroma, between, and occasionally extending into the lamella.

The keratocytes have a flattened cell body, a large eccentric nucleus and long branching processes which form contact with other cells in the same layer but do not form a syncytium. It is believed that these cells produce ground substance and collagen fibrils during embryogenesis and after injury. The wandering cells of the stroma migrate from the marginal loops of the corneal blood vessels.

DESCEMET'S MEMBRANE (POSTERIOR ELASTIC LAMINA)

It is a strong homogenous layer which binds the stroma posteriorly. It represents the basement membrane of the endothelium from which it is produced. Though elasticity is one of its physical characteristics, it is made up of collagen and glycoprotein with no elastic fibers visible by electron microscopy. Its thickness varies with age being 3µm at birth and 10 – 12 µm in young adults. It is very resistant to chemical agents, trauma, infection and pathological processes. Even when whole of the stroma is sloughed off, the descemet's membrane can maintain the integrity of the eyeball for a long. Further, unlike bowman's membrane when destroyed, it can regenerate. Normally it remains in a state of tension and when torn it curls inwards on itself. In the periphery it appears to end at the anterior limit of the trabecular meshwork as schwalbe's line.

ENDOTHELIUM

It consists of single layer of flat polygonal cells which on slit lamp biomicroscopy appear as mosaic. The cell density of endothelium is around 6000 cells/mm^2 at birth. The cell count falls by about 26% in the first year and a further 26% is lost over the next 11 years. Therefore,

with increasing age, the number of cells is reduced to 2400 – 3000cells/ mm^2 in young adults. The deficit left by the dying cells is filled by enlargement (polymegathism) of the remaining cells. Hence, these cells vary in diameter from 18 – 20 μ early in life to 40 μ or more in the aged.

There is a considerable functional reserve for the endothelium. Therefore, corneal decompensation occurs only after more than 75% of adult age cells are lost (ie. when the endothelial cell count becomes less than 500 cells/mm^2). The endothelial cells are attached to the descemet's membrane by hemidesmosomes and laterally to each other by tight junctional complexes. The desmosomal linkages and zonulae occludentes are continuous around the entire cell and thus close the intercellular space form the anterior chamber.

This linkage is calcium dependant and plays an important role in maintaining the barrier function of the endothelium. The endothelium also contains an active pump mechanism and is involved in active secretion and protein synthesis.

The high metabolic activity and energy production for the above process by the endothelial cells is evidenced by the presence of abundant mitochondria, free ribosome's, rough and smooth surface

endoplasmic reticulum and golgi complexes in the cytoplasm of these cells. In the eye, next to photo receptors, the endothelial cells contain highest number of mitochondria.

SOURCES OF NUTRIENTS

OXYGEN

The epithelium derives oxygen mainly from atmosphere through the tear film (active process) as well as through the limbal capillaries. The oxygen required by the epithelium is about one –tenth of that available from the atmosphere when the eyes are open and about one-fourth of that available from the palpebral conjunctiva when the eyes are closed.

The endothelium derives most of its required oxygen from the aqueous humour which has an oxygen tension of 72mmHg.

The major metabolic pathways in the cornea are Glycolysis and Kreb's cycle.

METABOLISM OF CORNEA

The cornea among other activities requires energy for maintenance of its transparency and dehydration. Energy in the form of ATP is generated by the breakdown of glucose. The most actively metabolising layers of the cornea are epithelium and endothelium, the former being ten times thicker than the latter thus requiring a proportionally larger supply of metabolic substrates.

PATHOGENESIS OF CORNEAL NEOVASCULARISATION

The factors that keep the cornea avascular are unknown and so is the pathogenesis of corneal vascularisation. However various theories which have been proposed largely agree on the role of chemical and mechanical factors in producing corneal neovascularisation.

Chemical Theory

There may be presence of vasostimulatory (VSF) factor or the breaking down of previously existing vasoinhibitory factor (VIF).

Role of Vasoinhibitory Factor

The idea that avascularity of the cornea might be due to the presence of a vasoinhibitory factor preventing vascular invasion was put forward by Meyer and Chafre.

Angiogenic stimulators and inhibitors are the counterbalancing systems that tightly control corneal angiogenesis. The healthy cornea is devoid of vascular elements and is maintained as an immune-privileged site.

This immunity is thought to be due to the plethora of antiangiogenic factors present in this tissue. These include pigment epithelium–derived factor (PEDF), maspin, thrombospondin, endostatin, a proteolysis product of type XVIII collagen; and angiostatin, a proteolysis product of plasminogen.

The class B scavenger receptor CD36[2] is a transmembrane glycoprotein that has been identified as the critical receptor for thrombospondin (TSP)-1, a potent endogenous inhibitor of angiogenesis, including that which occurs in the cornea. CD36 also binds to a variety of other ligands including oxidized low- density lipoproteins, oxidized phospholipids, *Plasmodium falciparum*–infected erythrocytes, collagen, and apoptotic cells.

Expression of CD36 is broad and encompasses micro vascular endothelial cells, monocytes/macrophages, platelets, conjunctival dendriform cells, and the retinal pigment epithelium. Furthermore, CD36 has been implicated in a wide variety of normal and abnormal biological functions, including angiogenesis, atherosclerosis, phagocytosis, inflammation, lipid metabolism, and removal of apoptotic cells.

With respect to its angiostatic functions, CD36 is essential for inhibiting in vitro endothelial cell migration and the formation of capillary-like structures by TSP-1. CD36 plays a critical role in vivo, as demonstrated by the inability of TSP-1 to inhibit angiogenesis in CD36 null mice. It has also been recently demonstrated the specific involvement of CD36 and TSP-1 in mediating anti- angiogenic signals in ischemic proliferative retinopathy[3].

Nevertheless, the involvement of CD36 and the relative role of its less well-characterized oxidized lipid-binding site in regulating pathologic corneal angiogenesis have not yet been fully elucidated[4].

Role of Vasostimulatory Factor

Campell and Michaelson 1949 using experimental corneal burns in animals postulated the release of vasostimulatory factor at the site of leision which diffuses through the stroma to the limbus and stimulates new vessel growth from the limbal plexus. It has also been postulated that hypoxia also induces neovascularisation by activation of vasostimulatory factor.

Mechanical Theory

Cogan postulated that blood vessel cannot invade normal cornea because of its compact structural nature and that loosening of the compactness of corneal tissue due to oedema was mandatory for neovascularisation. This mechanical theory was agreed to by many workers. However, Langham doubted the adequacy of oedema alone being responsible for neovascularisation. Clinically also it has been seen that in Fuch' s dystrophy and aphakic bullous keratopathy, it is rare for the vascularisation to occur even when the oedema extends to limbus.

Combined Mechanical and Chemical Theory

Maurice et al have demonstrated that both release of some vasostimulatory factor and loosening of compact corneal stroma by oedema are necessary for the neovascularisation to occur.

ROLE OF LEUCOCYTES IN CORNEAL NEOVASCULARIZATION[2]

There presently exists much experimental evidence supporting the hypothesis that leucocytes play a crucial role in corneal vascularisation. A comparative study was conducted on a wide spectrum of experimental models of corneal vascularisation including exposure of the cornea to noxious agents as alloxon, Sodium hydroxide and silver nitrate. Corneal vascularisation was also studied following the intracorneal injection of antigen into sensitized animals, as well as after the maintenance of animals on riboflavin deficient diets. In all these studies they observed that a leucocytic infiltration preceded the onset of vascular invasion. The various models consistently displayed three distinct phases with respect to the presence of leucocytes and blood vessels within the corneal stroma.

PREVASCULAR PHASE

Initially only leucocytes were observed in each avascular cornea.

VASOPROLIFERATIVE PHASE

Prevascular phase was followed by a time span when both proliferating capillaries and leucocytes were observed in the cornea simultaneously.

ESTABLISHED PHASE

During this phase the leucocytes totally disappeared from the corneal stroma but the blood vessels remained.

VASCULAR ENDOTHELIAL GROWTH FACTOR

1. VEGF regulation and receptors

Vascular endothelial growth factor (VEGF) or vascular permeability factor is a secreted growth factor peptide of 45 kDa homodynamic glycoprotein that functions as an endothelial cell- specific mitogen and vasopermeability factor. It is activated by hypoxia, protein kinace C activation, advanced glycation products, reactive oxygen species, activated oncogens and variety of cytokines[6].

Activation of VEGF induces proteolytic activities, endothelial cell proliferation, endothelial cell migration, capillary tube formation, induces vascular permeability, and inhibits apoptosis.

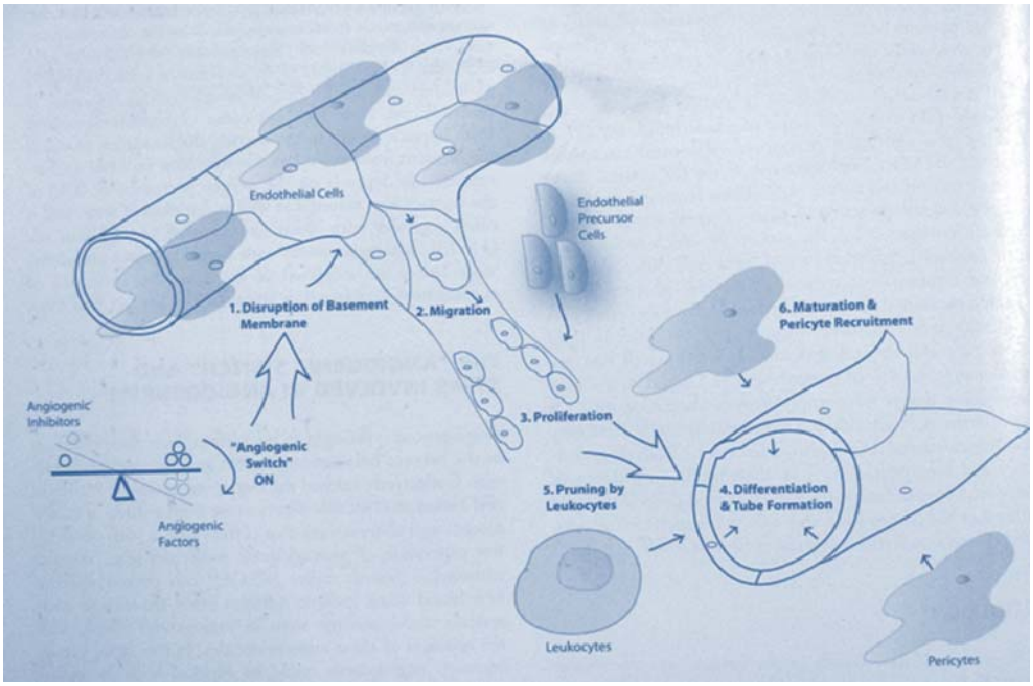


Fig 3. Stages of vascularization

In angiogenesis VEGF mediates the initial five steps

i. disruption of basement membrane

ii. migration

iii. proliferation

iv. differentiation and tube formation

v. pruning by leucocytes

Whereas platelet derived growth factor mediates maturation and pericyte recruitment

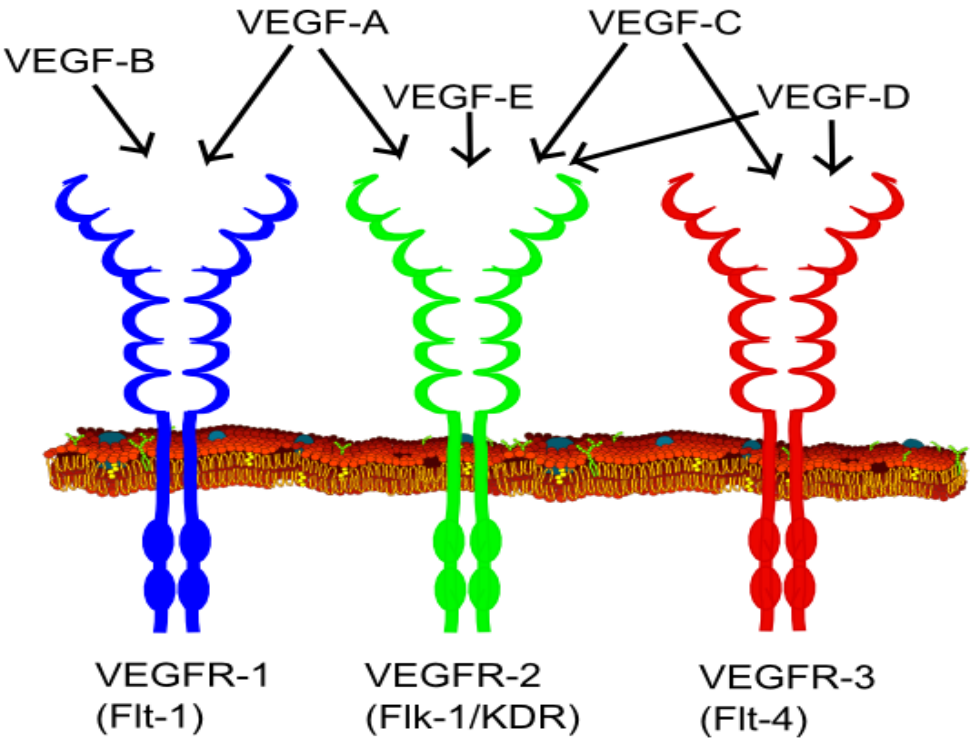


Fig 4. .VEGF receptors

Genetic knock out of the only a single VEGF allele leads to embryonic lethality demonstrating a critical contribution for VEGF in embryonic vasculogenesis. Interestingly modest over expresssion of VEGF also proved lethal to the embryo. These VEGF levels must be closely regulated for development to proceed normally. VEGF acts through binding through two receptor kinases, VEGF R-1, VEGF R-2 which respond in typical fashion to ligand binding by activation of single transduction cascades.

VEGF R-2 is principally responsible for mediating effects of VEGF on angiogenesis and vascular permeability. VEGF R-1 has been implicated in mediating chemotaxis to VEGF, a process that may contribute to pathogenic angiogenesis, ransduction of matrix metalloprpteinases and release of hepatic pancreatic factors. Its functions may also include negative regulators of VEGF by sequestering it thereby making it less available to VEGF R-2.[22]

The VEGF molecular family consists of five members

- 1.Placental growth factor
- 2.VEGF A
- 3.VEGF B
- 4.VEGF C
- 5.VEGF D

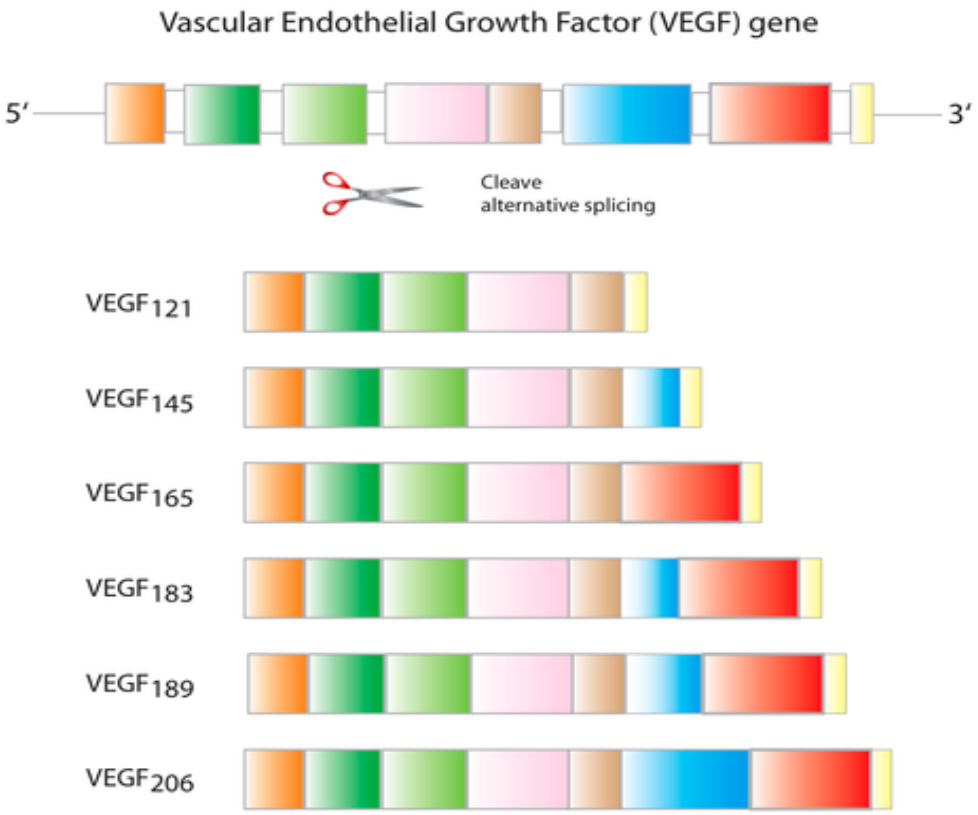


Fig. 5. Vascular Endothelial Growth Factor Gene

There are at least 6 major known isoforms of VEGF that arise from alternate splicing of mRNA of a single gene; VEGF115, VEGF121, VEGF145, VEGF165, VEGF189, and VEGF 206. VEGF165 is the predominant pathological isoform[7]. It plays a major angiogenic role in several ocular pathologies characterized by NV. It was recently shown that VEGF was up-regulated in inflamed and vascularised corneas in humans and animal models.

VEGF-A is expressed by macrophages in the inflamed cornea and is down regulated after activation of CD36. It is well known, however, that transmigrating and invading macrophages are closely associated with neovascularization and provide much of the requisite VEGF that drives this process .VEGF can be inhibited by blocking its production or receptors or the molecule itself in the extra cellular space.

VEGF AND SYSTEMIC DISEASES[8]

VEGF stimulated collateral blood vessel formation helps to preserve myocardial function during coronary arterial occlusion. Several observations including the fact that direct VEGF transfer therapies have proved to be effective in coronary heart disease as well as peripheral vascular disease suggests that VEGF plays a significant role in this adaptive process. Thus although anti VEGF therapies appear promising as a means of reducing neo vascular complications from ischemic ocular diseases, they also have a potential to decrease collateral vascular formation and thereby increase micro vascular complications associated with myocardial infarction and peripheral limb ischemia.

Over expression of VEGF has been observed in a variety of cancers and has been associated with a worse relapse free and over-all survival.

VEGF and RETINAL VASCULAR DISEASE

Levels of ocular VEGF are tightly correlated with both growth and permeability of new vessels. In patients with proliferative diabetic retinopathy in which tissue hypoxia promotes neovascularisation; levels of VEGF are elevated in ocular tissues. These elevated levels of VEGF decline when treatment with laser photocoagulation induces regression of neovascularisation. Elevated VEGF levels are seen also in patients with Branch and Central retinal vascular occlusions.

VEGF IN HUMAN OCULAR FLUIDS

The growth of neovascular vessels in response to retinal ischemia (factor X) was initially proposed by Michaelson almost half a century ago. More recently, corneal hypoxia and inflammation, which has been implicated in the pathogenesis of corneal neovascularization, was shown to cause increased expression of VEGF. Indeed, evidence suggests that VEGF is a major mediator of corneal neovascularization. In 1994,

Aiello and colleagues demonstrated high levels of VEGF in ocular fluid of patients with diabetic retinopathy and other vascular disorders. Further work by Aiello found that suppression of retinal neovascularisation was achievable in vivo by inhibition of VEGF using soluble VEGF receptor chimeric proteins.

VEGF IN HUMAN CORNEAL TISSUE

Philipp et al. Have shown that VEGF is expressed in all three layers of inflamed cornea. Including the epithelium, stroma, and endothelium. It is highly expressed in the vascular endothelial cells of limbal vessels and in newly formed vessels in the stroma and weakly in keratocytes. Therefore subconjunctival injection route will be better for the access of medication to limbal endothelial cells[1].

PLATELET DERIVED GROWTH FACTOR

- PDGF-B is a growth factor structurally related to VEGF
- The contributions of PDGF-B to angiogenesis are mediated largely through its effects on mural cells such as pericytes and vascular smooth muscle cells

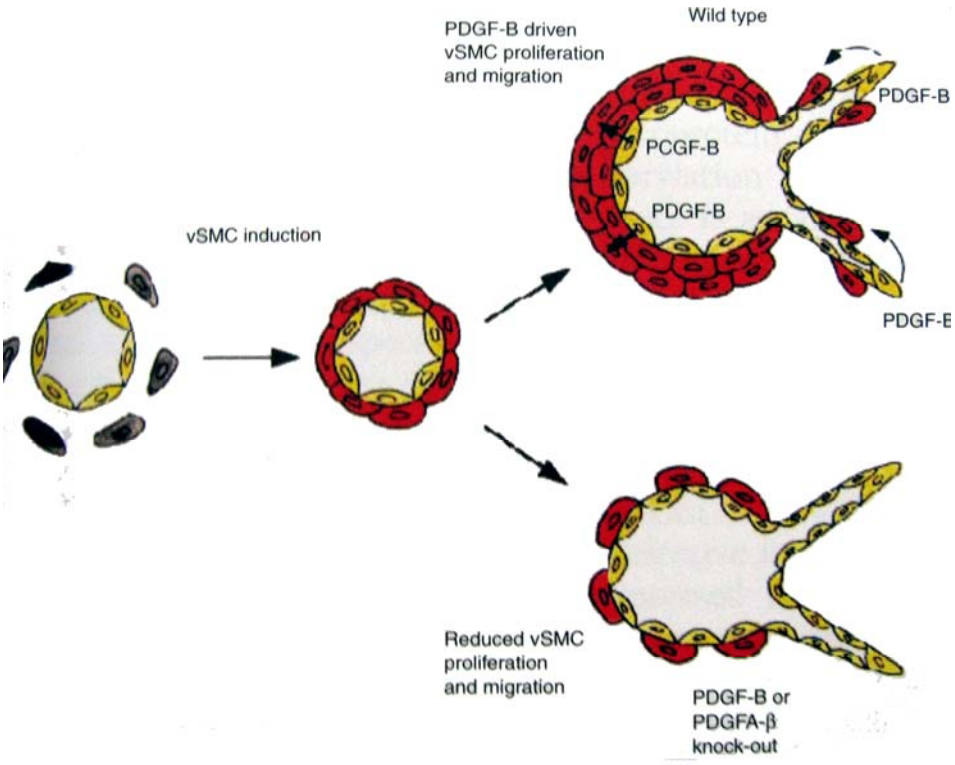


Fig. 6. Role of PDGF in corneal neovascularization

- Mural cells are recruited to the endothelium primarily in response to endothelial cells

- Mural cells are recruited to the endothelium primarily in respose to endothelial cell-secreted PDGF-B, which activates receptors on the mural cells and stimulates their migration and proliferation.

- Vasculature that is stably covered with mural cells is largely resistant to the effects of VEGF withdrawal, making it less susceptible to therapeutic intervention with VEGF blocking agents.

- Blocking VEGF and PDGF may provide improved efficacy in the treatment of established ocular neovascular lesions

VEGF INHIBITION IN CORNEAL NEOVASCULARISATION

In December 2004, Pegaptanib sodium became the first anti-VEGF agent approved by the United States Food and Drug Administration (USFDA) for treatment of all neovascular AMD regardless of lesion composition

Pegaptanib (Macugen, Eyetech/Pfizer, Inc, New York, NY), is a pegylated aptamer that consists of an RNA oligonucleotide ligand that binds human VEGF 165, with high affinity and specificity. Pegaptanib does not block the other isoforms of VEGF.

Bevacizumab is a full length, recombinant, humanized monoclonal antibody, directed against all VEGF isoforms. It was the first anti-VEGF agent approved by the FDA for systemic administration in treatment of colorectal cancers[8]. Unfortunately, systemic effects, such as, elevation of systolic blood pressure and the potential for systemic thromboembolic events were concerns in the cancer studies, and although the first case using systemic bevacizumab for AMD held promise, the potential for adverse effects was deemed risky.

In the summer of 2005, Rosenfeld pioneered the use of intravitreal fractionated dose of bevacizumab for retinopathy in AMD with impressive results. After that many experimental studies conducted on animals by artificially inducing neovascularization.

RATIONALE FOR ANGIOGENESIS INHIBITORS IN CORNEAL NEOVASCULARIZATION

Animal studies have supported the role of VEGF in the pathogenesis of corneal neovascularisation. In expermental models, increased levels of VEGFmRNA and protein and VEGF receptors have been demonstrated in corneal neovascularization. Human corneal buttons with New vessels have been shown to have higher levels of VEGF and its receptors.VEGF inhibition therefore supress corneal neovascularization and help improve vision or increase the chance of transplant survival. Corneal neovascularization was induced in animals by following these three models,

Corneal Micropocket Neovascularization Models

In brief, an aliquot of bFGF or VEGF was added to 2 μL of 12% hydron polymer (poly-HEME) in ethanol. Four-microliter aliquots of the mixture were placed on parafilm (American National Can, Greenwich,) and allowed to dry to produce pellets, each containing 500 ng of bFGF or 100 ng of VEGF. A partial corneal incision was made at the limbus, and dissection to the corneal stroma was performed up to 4 mm from the limbus. The pellet was placed in this pocket[2].

Alkali Induced-Corneal NV Model

Induction of the alkali-induced corneal NV model was performed as described by Ormerod et al with some modifications. In brief, a 5.5-mm-diameter circular filter disc was incubated with 20μL of NaOH for 60 seconds. The filter disc was then placed on the cornea surface of anesthetized rabbits under the surgical microscope for 30 seconds. The ocular surface was then irrigated with 25 ml of physiological saline[2 9].

Limbal Injury–induced Corneal NV Model

The limbal injury–induced corneal NV model was produced by surgical removal of the circumferential limbal tissue 2 mm into the cornea and 3 mm into the conjunctiva. Superficial lamellar keratectomy was also performed to remove the entire corneal epithelium. In this model, corneal NV occurred within 2 weeks after surgery[10 11].

These studies have proven the effectiveness of subconjunctival and topical bevacizumab on corneal neovascularisation by observing the regression of new vessels.

Ranibizumab, (Lucentis, Genentech Inc, San Francisco) is a fragment of a humanized monoclonal antibody directed towards all isoforms of VEGF-A. It has a molecular weight of 48KD and is produced by an E. coli – expression system. Its unique structure was specifically engineered for ocular diseases. Ranibizumab is made up of just the Fab fragment that was the basis for the full length antibody, bevacizumab and has been affinity matured to have a higher binding affinity for VEGF than bevacizumab has[12].

The binding of Ranibizumab to all isoforms of VEGF-A prevents dimerisation with the VEGF receptors on cell surfaces (VEGF R1 and VEGF R2), thus reducing vascular leakage, angiogenesis and endothelial cell proliferation.

Both Ranibizumab and bevacizumab block all forms of VEGF. Nevertheless, they have distinct differences that offer each advantage over the other. One advantage of Ranibizumab is that, it lacks the Fc region of the antibody, making it less likely to cause complement mediated inflammation after injection. Ranibizumab has only one binding site for VEGF, while bevacizumab has two.

AN UPDATE ON BEVACIZUMAB

Bevacizumab (Avastin, Genentech) is a full length humanized murine monoclonal antibody, directed against all the biologically active forms of VEGF – A. Bevacizumab, the first anti – VEGF drug to be approved by the FDA was developed as an intravenous therapy for cancer patients, because VEGF is one of the major angiogenic stimuli, responsible for neovascularisation in tumours[12].

Anti – VEGF therapy has shown promising results in several forms of cancer but the drug is currently approved only for the treatment of metastatic colorectal cancer. When used in cancer therapy, bevacizumab is infused at a dose of 5 mg/kg body weight, every 2 weeks until the patient dies or significant disease progression is observed. In clinical trials, the most common adverse event caused by bevacizumab was hypertension[6&12].

The first two agents Pegaptanib and Ranibizumab have been approved by the FDA for use in neovascular AMD. The third drug bevacizumab which is a full-length humanized antibody against VEGF, has been approved for use in oncology but is also widely used off-label to treat choroidal neovascularization, central retinal vein occlusion,

proliferative diabetic retinopathy, and iris neovascularization with encouraging results. Bevacizumab has now been widely adopted and is arguably part of the standard of care for the treatment of neovascular AMD for many patients[12].

PHARMACOKINETICS OF INTRAVITREALLY ADMINISTERED BEVACIZUMAB

In the retina/choroid and iris/ciliary body after intravitreal injection, bevacizumab concentration above IC_{50} was maintained for approximately 11.7 and 10.3 weeks, respectively, whereas that above 500 ng/mL was maintained for 7.7 and 6.6 weeks. A recent report regarding long-term follow-up results for intravitreal bevacizumab treatment of neovascular age-related macular degeneration has described the mean number of injections during 1 year as 3.4. Thus, a single intravitreal injection of bevacizumab is active for approximately 3 months. Many studies in rabbits showed bevacizumab concentration in the retina/choroid after intravitreal injection were maintained for approximately 3 months (11.7 weeks) in the effective range and were consistent with reinjection intervals in clinical situations.[13]

PHARMACOKINETICS OF SUBCONJUNCTIVALLY ADMINISTERED BEVACIZUMAB

In general, drug injected into subconjunctival space has two fates: direct trans- scleral delivery into intraocular tissues or clearance via conjunctival blood and lymphatic flow. Since IgG has a relatively high scleral permeability and has the same molecular weight as bevacizumab, some of the bevacizumab injected into the subconjunctival space may penetrate intraocular tissues including the retina/choroid, iris/ciliary body, and vitreous via the sclera. The bevacizumab level in the retina/choroid and iris/ciliary body was maintained above IC_{50} for 8.6 and 8.4 weeks, respectively, whereas it was maintained above 500 ng/mL for 0.3 weeks in the iris/ciliary body. The sclera consists of collagen and elastin chains that create a fiber matrix in which the pore diameter and intracellular space may determine the permeability of drugs. Negatively charged drugs have been found to have higher permeability than those with positive charges in bovine and porcine sclera. Proteoglycans in the sclera are negatively charged, which may contribute to the binding of positively charged molecules. An isoelectric point of bevacizumab has been found to be approximately 8.4. Therefore, longer $T_{½}$ in the iris/ciliary body and retina/choroid after subconjunctival injection compared with those after intravitreal injection

may sustain bevacizumab delivery into intraocular tissues due to scleral depot binding of bevacizumab to the scleral matrix.[4&13]

Since the conjunctival blood vessels do not form a tight junction barrier, bevacizumab can enter into the blood circulation by pinocytosis and/or convective transport through paracellular pores in the vascular endothelial layer. The Fc receptor, which binds to both albumin and the Fc portion of IgG, was detected in the lymphatic vessels but not in the blood vessels of the conjunctiva. It may be that the function of the Fc receptor in the conjunctival lymphatic vessels is to act as an efflux receptor for the efficient elimination from the conjunctival space. Residual bevacizumab, other than the bevacizumab that directly permeated the sclera and was introduced into the blood circulation, may be eliminated from the conjunctival tissue into the lymphatic vessels via convective transport with lymphatic fluid.

Systemic exposure of bevacizumab when administered by intravitreal and subconjunctival injection was very similar. Bevacizumab was detected in the fellow eyes treated by intravitreal or subconjunctival injection. Bevacizumab may have been transported through the systemic circulation into the fellow eyes. In the fellow eyes

treated by intravitreal injection, bevacizumab concentration in the retina/choroid was maintained above IC_{50} for 8.0 weeks.

Heiduschka et al. demonstrated that bevacizumab can penetrate the retina and is transported into the retinal pigment epithelium, choroid, and photoreceptors after intravitreal injection using an immunohistochemical staining assay with donkey anti-human IgG. In that study, the antibody used for the detection of bevacizumab recognized the injected bevacizumab molecule specifically[10].

The strong intracorneal bevacizumab staining could persist for at least 3 days in the VEGF micropocket and alkali-induced corneal NV models after a single subconjunctival injection. The distribution of intracorneal bevacizumab could persist at least up to 7 days in the normal corneas. The reason for the different durations of intracorneal diffusion remains to be evaluated. Although not proven, the shorter duration of bevacizumab distribution in the vascularized corneas may be caused by the rapid absorption through the corneal vessels. In some studies, the eyes treated with bevacizumab showed significant decrease in the amount of neovascularization especially those treated with subconjunctival bevacizumab[2&3].

TOPICALLY ADMINISTERED BEVACIZUMAB

While there is substantial evidence for the intravitreous administration of bevacizumab in the treatment of choroidal NV, data regarding the safety and efficacy of topical bevacizumab in the treatment of corneal NV are, as yet, preliminary. Topical bevacizumab was demonstrated to inhibit corneal NV after chemical injury in an experimental rat model. In humans, a small number of studies showed that topical bevacizumab can reduce corneal NV in a few patients with significant corneal NV. However, many aspects of topically administered bevacizumab in the treatment of corneal NV, including long-term safety and efficacy against actively growing as well as established corneal NV, optimal dosing for modulating the neovascular process, and long-term stability of treatment results, have not been well known.

Although this reduction in neovascularization is significant, there is no complete regression of new vessel. This may be due to incomplete diffusion and absorption of the drug through the conjunctiva and due to washing out of the drug by the tear film and hence inadequate access to the ocular surface. Another reason is that bevacizumab inhibits only

VEGF and we know that fibroblast growth factor and other cytokines contribute to the pathology of neovascularization which cannot be inhibited by this drug. So inhibition of VEGF alone may not cause complete regression of the vessels, combination with steroids might cause complete regression of the vessels [15&16].

In the case of subconjunctival injection, studies showed that most of the bevacizumab in the treated eyes was derived from the systemic circulation, and so the retinal and choroidal pharmacokinetics profile in the fellow eyes was similar to that in the treated eyes. In the fellow eyes, bevacizumab concentration in the retina/choroid was maintained above IC_{50} (inhibitory concentration) for 5.2 weeks. In the iris/ciliary body, a similar profile was observed, but in the fellow eye, bevacizumab was not detected at 12 weeks after injection. Of interest, corneal deposition of bevacizumab for 4 weeks after subconjunctival injection was observed by immunohistochemistry. Therefore, subconjunctival bevacizumab may be a promising treatment, not only for neovascular diseases of the iris, retina, and choroid but also for corneal neovascularization.[4]

SIDE EFFECTS OF SUBCONJUNCTIVALLY ADMINISTERED BAVACIZUMB [13]

SYSTEMIC SIDE EFFECTS

There are several reports that have noted the systemic adverse effects of bevacizumab after intravitreal injection. These adverse effects are similar to the ones reported for intravenous administration of bevacizumab for cancer treatment, such as systemic hypertension, thromboembolic diseases and death. In addition, Shima et al. found irregular vaginal bleeding as a complication in young women receiving intravitreal injection of bevacizumab. It should be noted, however, that these were small studies with a small amount of participants. Systemic adverse effects of bevacizumab may occur after subconjunctival injection, as well as after intravitreal or intravenous injection. Therefore, intravitreal or subconjunctival bevacizumab should be used with caution in elderly patients with choroidal or iris neovascularization. However, efficacy in the fellow eye should be expected to derive from the systemic circulation.

OCULAR SIDE EFFECTS

Corneal epithelial defect, corneal ulcer, corneal oedema, Conjunctival necrosis, anterior uveitis, and Infectious endophthalmitis.

OTHER MODALITIES OF TREATMENT FOR CORNEAL NEOVASCULARISATION

Current treatments for corneal NV including medications, such as steroids or non-steroidal anti-inflammatory agents, laser photocoagulation, fine-needle diathermy, photodynamic therapy, or restoration of the ocular surface with the use of conjunctival, limbal, or amniotic membrane transplantation have demonstrated variable and largely limited clinical success.

The highly variable efficacy and myriad side-effects (cataract, glaucoma, and increased risk of infection) of topical and systemic corticosteroids are well known to clinicians who use these agents regularly in trying to arrest these disease processes. Other treatment modalities are often ineffective, or vessel recanalization occurs requiring multiple treatment sessions which can lead to serious side effects. Furthermore, none of these treatments specifically target the molecular mediators of angiogenesis.

COST OF BEVACIZUMAB

The cost of single vial of bevacizumab (100mg/4ml) is 700$

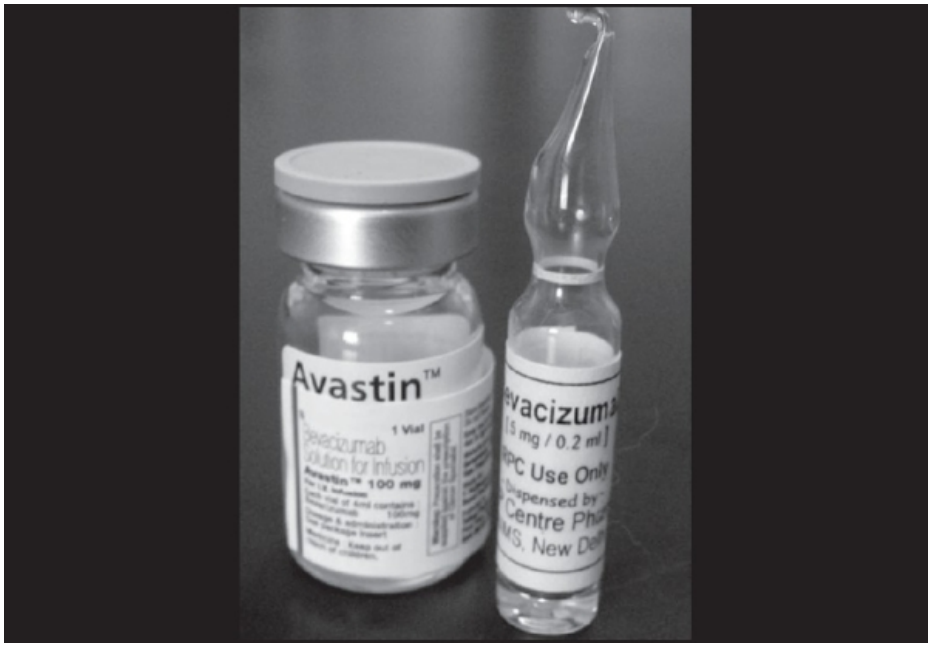


Fig. 7. Injection Avastin

OTHER USES OF SUBCONJUNCTIVAL BEVACIZUMAB

There are ongoing studies to prove the effectiveness of subconjunctival injection of bevacizumab in managing

- ✓ highly vascular neoplasm of the conjunctiva such as conjunctival intra epithelial neoplasm (CIN)[20]
- ✓ highly vascularized pterygium AND
- ✓ preventing bleb failure after glaucoma surgeries

PART II

AIM OF THE STUDY

- To study the effect of subconjunctival Bevacizumab on corneal neovascularization in patients with

1. Leucomatous opacity following exanthematous fever and post hydrops
2. Pseudophakic bullous keratopathy
3. Healed corneal ulcer post trauma and infection
4. Previously failed Optical keratoplasty

- To evaluate regression of new vessels of cornea
- To evaluate the safety and efficacy of subconjunctival injection of Bevacizumab
- To evaluate the outcome of OKP after subconjunctival Bevacizumab

MATERIALS AND METHODS

This study was done in Cornea Department Regional Institute Of Ophthalmology And Government Ophthalmic Hospital Chennai during January 2009 To January 2010.

Twenty eyes of twenty patients with corneal neovascularization due to various pathologies (mentioned below) have been selected for the study.

STUDY DESIGN

- Interventional
- Prospective
- Non-Randomized clinical study

INCLUSION CRITERIA

VASCULARISED CORNEAS OF PATIENTS WITH

- Leucomatous opacity following exanthematous fever and post hydrops
- Pseudophakic bullous keratopathy
- Healed corneal ulcer post trauma and infection
- Previously failed Optical keratoplasty

EXCLUSION CRITERIA

- Patients with uncontrolled systemic hypertension with systolic blood pressure of ≥150mm Hg or diastolic blood pressure of ≥90 mm of Hg
- Patients with recent Myocardial Infarction
- Patients with recent Cerebro Vascular Accidents
- Diabetes mellitus
- Renal, liver, and coagulation abnormalities including current anticoagulation medications
- Current or recent systemic corticosteroid therapy or periocular corticosteroids injections to the study eye
- Ocular or periocular malignancy
- Pregnancy, lactating women, postmenopausal women not using adequate contraception
- Any condition that precluded patients ability to comply with study requirements including completion of study

PROCEDURE

Patients who were referred to cornea clinic with corneal neovascularization (mentioned above in inclusion criteria) were selected for the study.

A brief history of all the patients were taken and subjected to detailed ocular and systemic examination.

Anterior segment examination was done with slit lamp biomicroscopy. Standardized corneal photographs were taken with 10X magnification with slit lamp biomicroscopy using digital camera.

The off-label use of the drug and its benefits were discussed with all the patients. Informed consent was obtained. Detailed corneal drawings were done.

NUMBER OF PATIENTS

Twenty eyes of twenty patients with corneal neovascularization due to various pathologies (mentioned above) have been selected for the study.

- Five eyes of 5 patients with healed fungal corneal ulcer

- Two eyes of 2 patients with Leucomatous opacity post exanthematous fever
- Seven eyes of 7 patients with pseudophakic bullous keratopathy
- Three eyes of 3 patients with previous failed keratoplasty due to vascularization
- One eye with post hydrops (keratoglobus)
- Two eyes of 2 patients with post traumatic leucomatous opacity

The off-label use of the drug and its potential risks and benefits were discussed extensively with all patients. All patients signed a comprehensive consent form before administration of the subconjunctival injection of bevacizumab.

A commercially available bevacizumab (2.5mg/0.1ml, 100 mg/4 mL) was prepared for each patient and placed in a tuberculin syringe using aseptic techniques.

Topical anesthetics (4% xylocaine/ paracaine eye drops) and antibiotics are applied

- The eye had been prepared with 5% povidone iodine in a standard fashion
- Then draped with an eye mask
- Opsite was applied
- Using lid speculum eye was exposed
- Bevacizumab (2.5mg/0.1ml) was injected subconjunctivally in the quadrant of vascularization 1cm from the limbus
- Patients were instructed to apply antibiotic eye drops for 3 days 4 times a day

FOLLOW-UP

Follow up visits were done on 14th day, 1st, 3rd, & 6th month post injection. All the 20 patients completed 6 months of follow-up. At each visit patients were checked for regression of new vessels i.e. reduction in both number and caliber of the vessel. Patients were also examined for signs of graft rejection (those who have been operated) and side effects.

MAIN OUTCOME MEASURES

A. Regression of corneal new vessels

B. Efficacy and safety of subconjunctival Bevacizumab

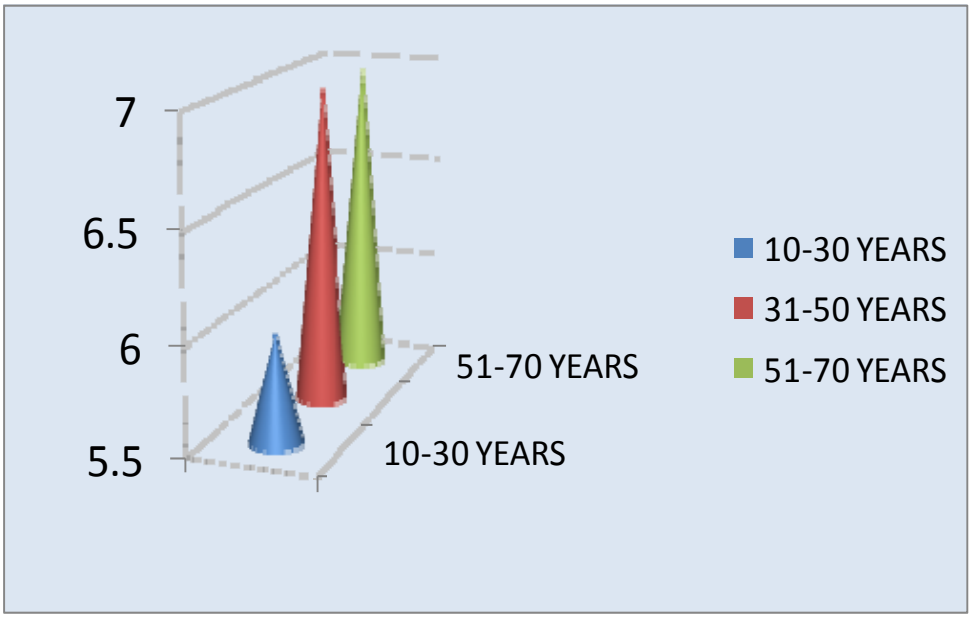
C. Outcome of OKP after subconjunctival Bevacizumab injection

OBSERVATIONS AND RESULTS

TABLE 1; AGE DISTRIBUTION

Total number of patients – 20

Age distribution	No of patients	Percentage (%)
10 – 30 yrs	6	30
31 – 50 yrs	7	35
51 – 70 yrs	7	35



In our study the upper age limit being 70 years and lower age limit being 15 years. The mean age was 42 years.

TABLE 2; SEX DISTRIBUTION

Sex	No of patients	Percentage (%)
Male	14	70
Female	6	30

Of the 20 patients who met the inclusion criteria for the study, 16 were male and 4 were female.

MALE: FAMALE ratio ≈ 2.3:1

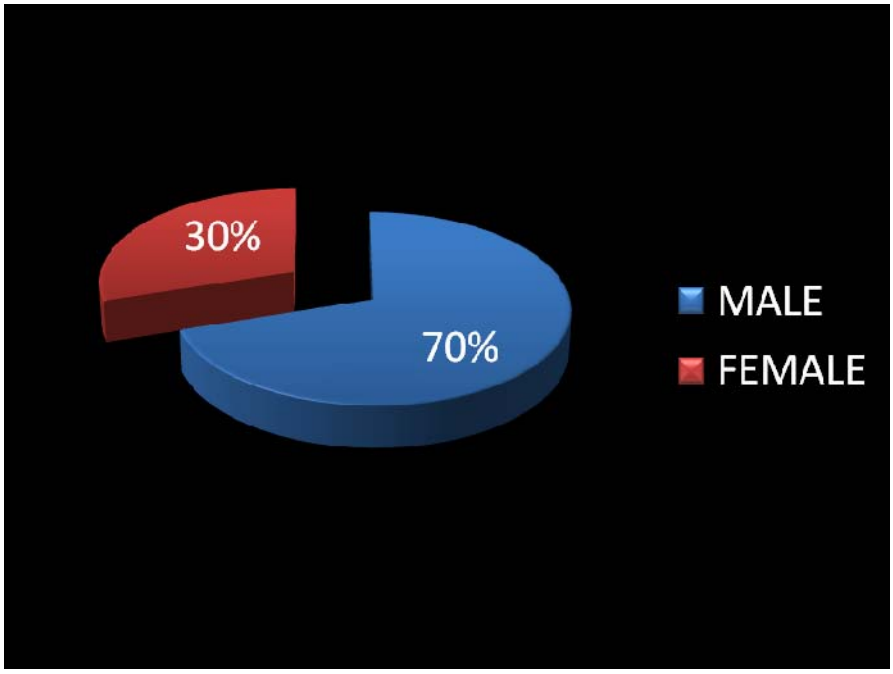
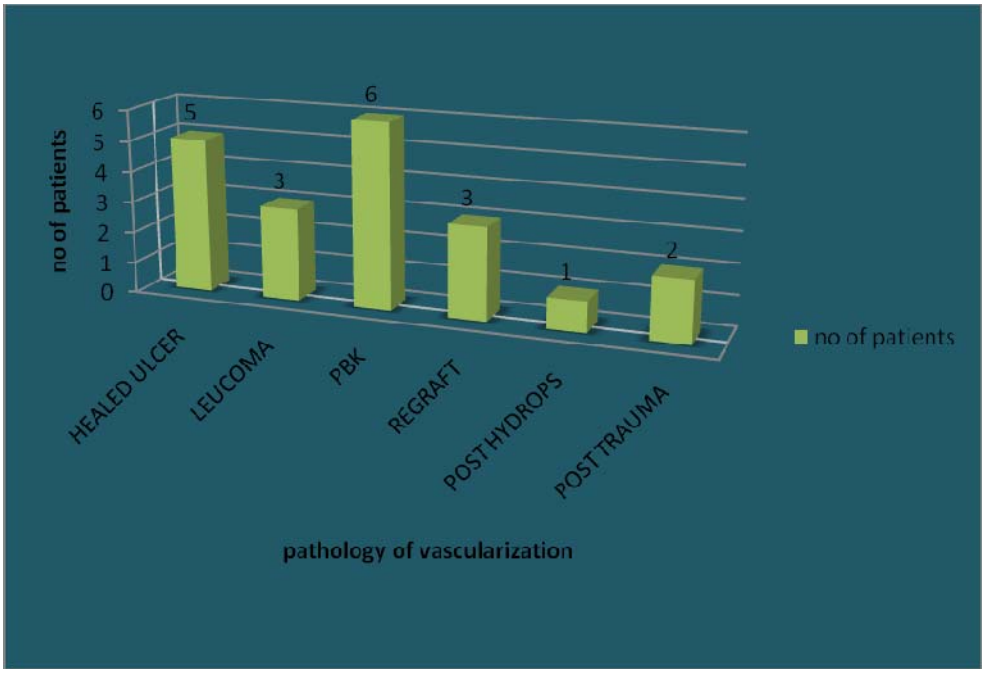


TABLE 3: INDICATIONS

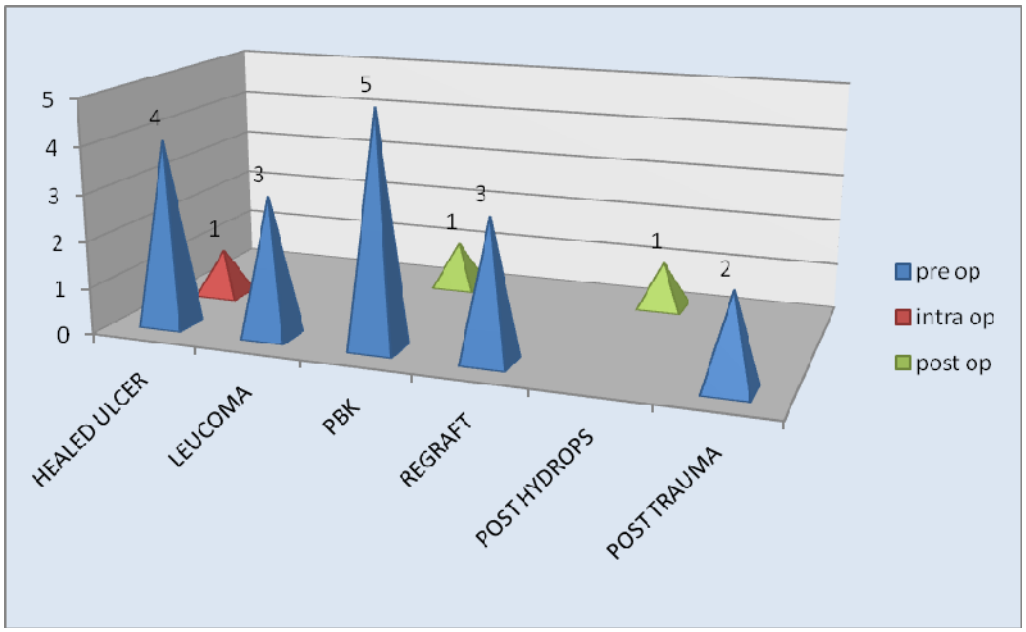
Total number of patients ─ 20

Indication	No of patients	Percentage (%)
Healed fungal corneal ulcer	5	25
Leucomatous opacity –post exanthematous fever	3	15
Pseudophakic bullous keratopathy	6	30
Previously failed OKP for regraft	3	15
Post hydrops - keratoglobus	1	5
Post traumatic leucomatous opacity	2	10



TABLE; 4 TIME OF INJECTION

Indication	Pre operative	Intra operative	Post operative
Healed fungal corneal ulcer	**4**	**1**	
Leucomatous opacity – post exanthematous fever	**3**		
Pseudophakic bullous keratopathy	**5**		**1**
Previously failed OKP for regraft	**3**		
Post hydrops - keratoglobus			**1**
Post traumatic leucomatous opacity	**2**		

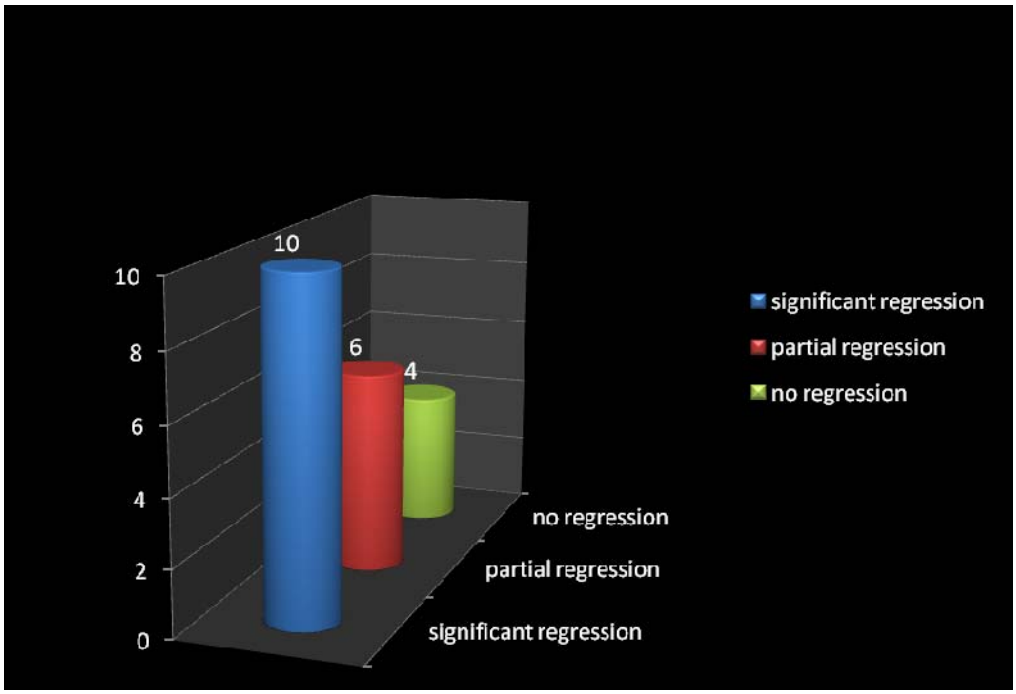


MAIN OUTCOME MEASURES

A. REGRESSION OF CORNEAL NEW VESSELS

Regression	No of patients	Percentage (%)
Complete regression	**10**	**50**
Partial regression	**16**	**30**
No regression	**4**	**20**

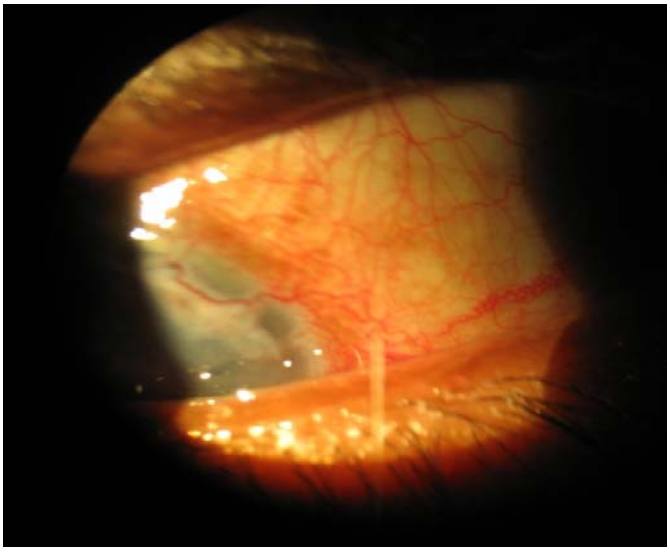
The reduction of corneal new vessels occurred to a different degree in each patient;



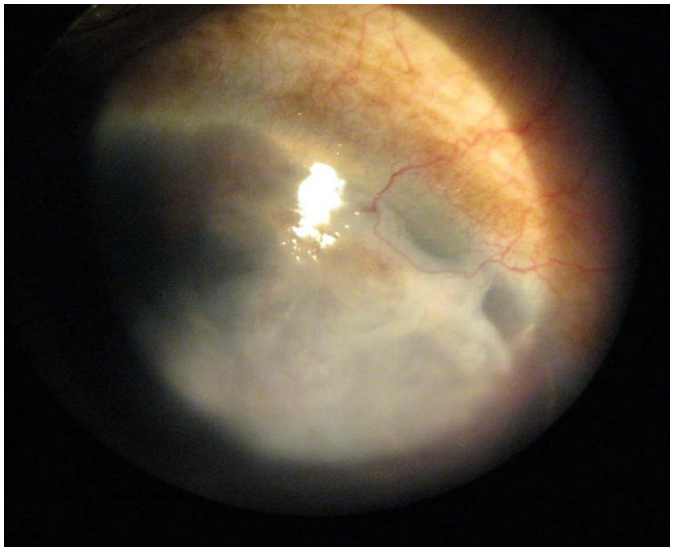
a. COMPLETE REGRESSION

CASE – 1

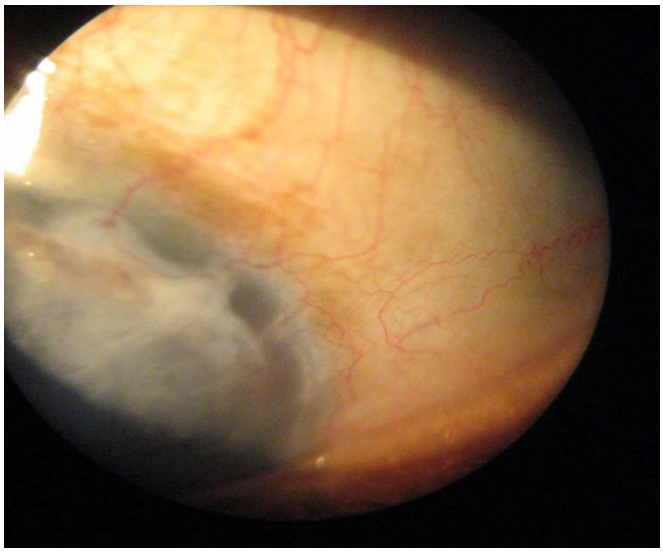
Leucomatous Opacity with Neovascularization
Pre –injection



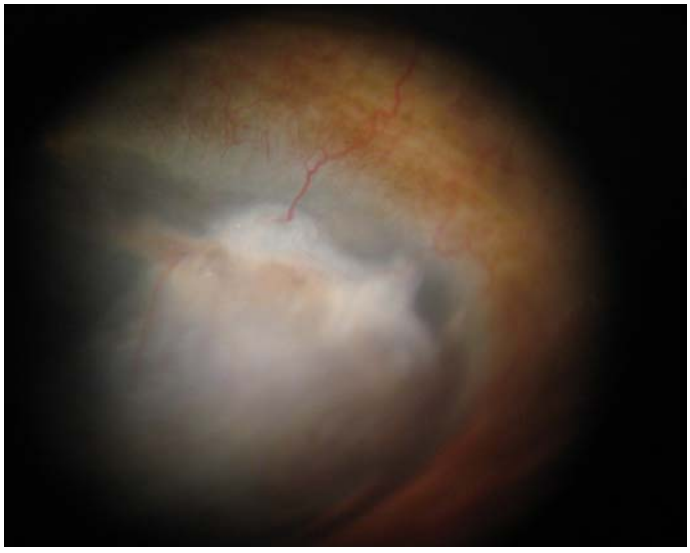
2 weeks post-injection



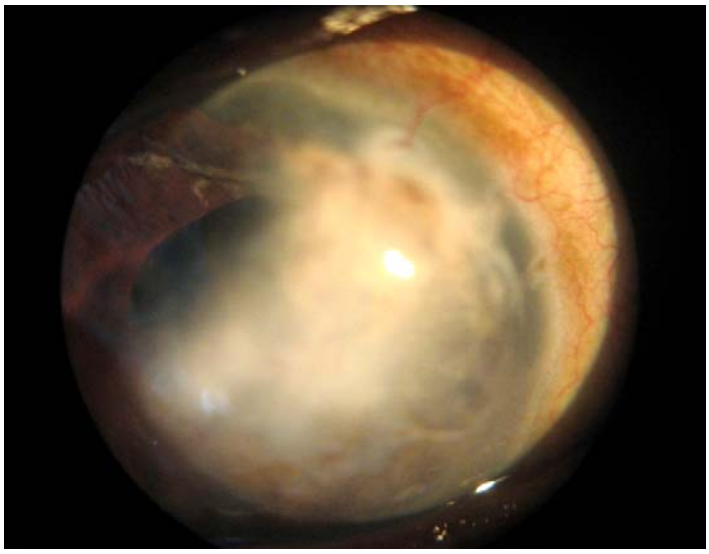
1 month post-injection



3rd month post-injection



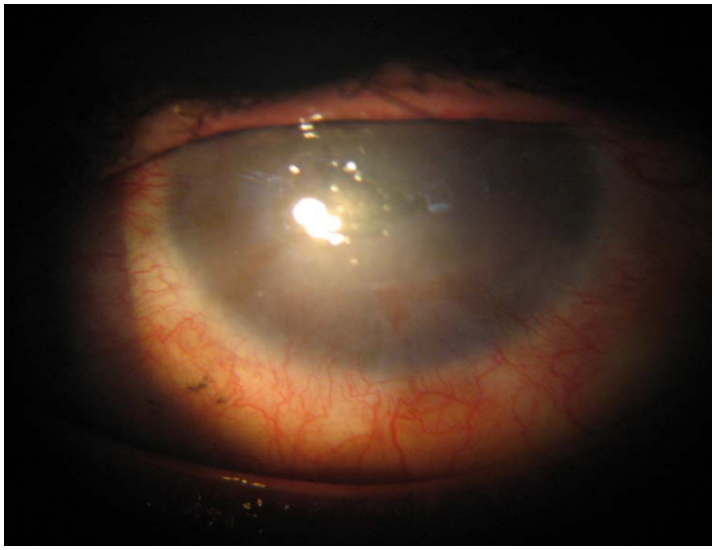
6th month post-injection



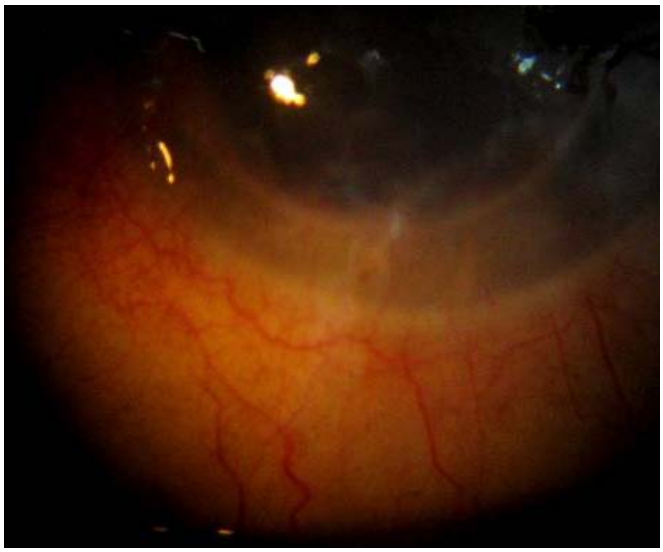
This patient showed almost complete regression of the new vessels at the end of 6 months.

CASE-2

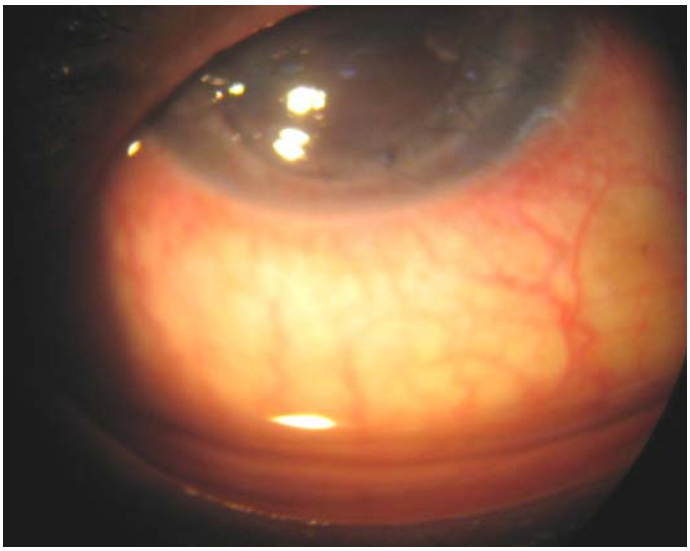
Healed corneal ulcer
Pre- injection



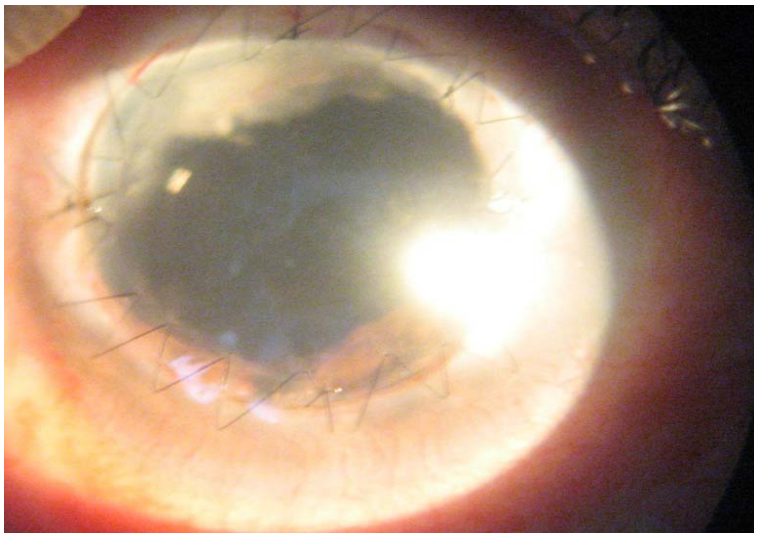
2 weeks post-injection



1 month post-injection

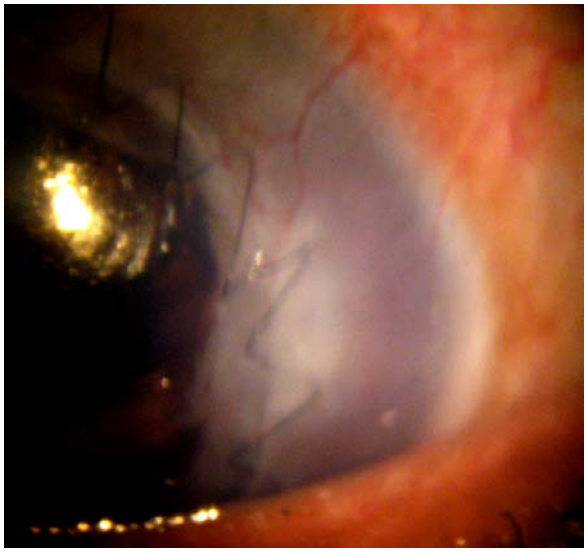


3 rd month post-injection

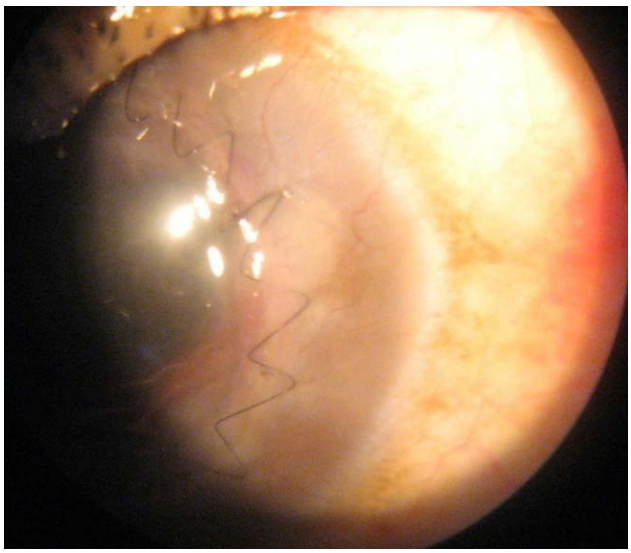


CASE-3

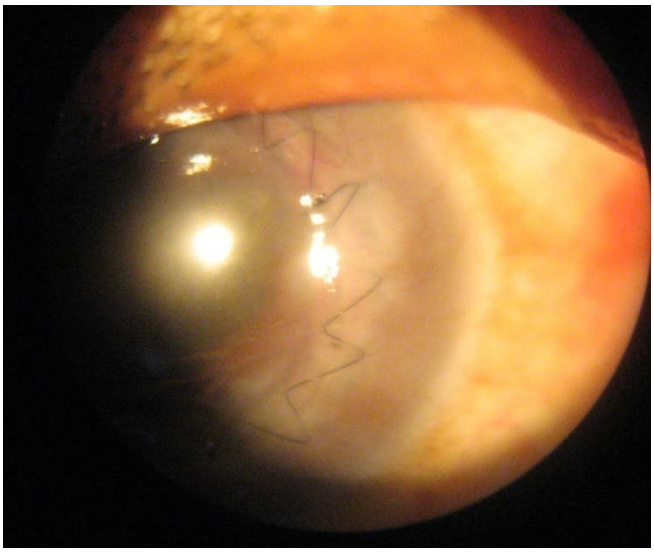
Healed corneal ulcer with leucomatous opacity
Injection given during keratoplasty



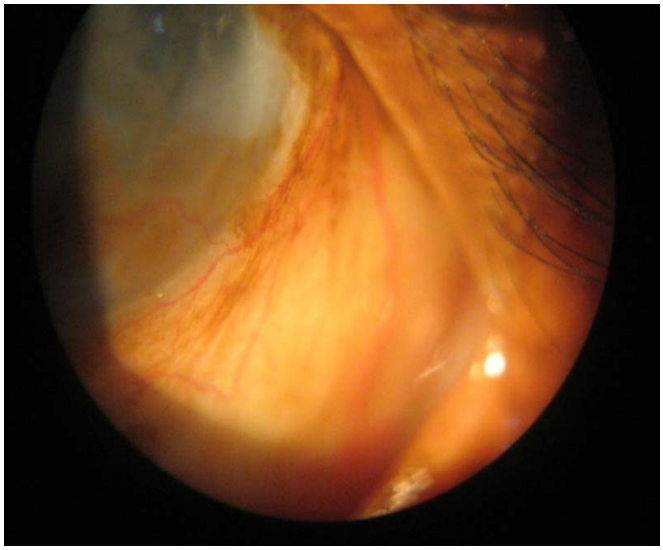
1 month post injection



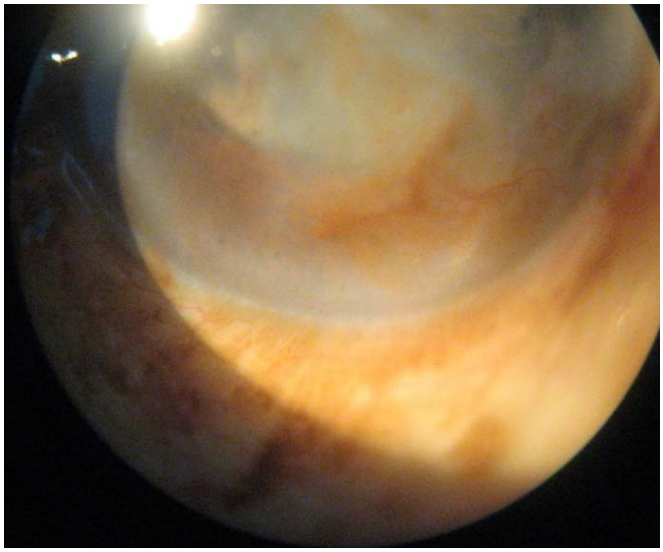
3rd month post-injection



CASE-4
Pseudophakic Bullous Keratopathy
Pre-Injection

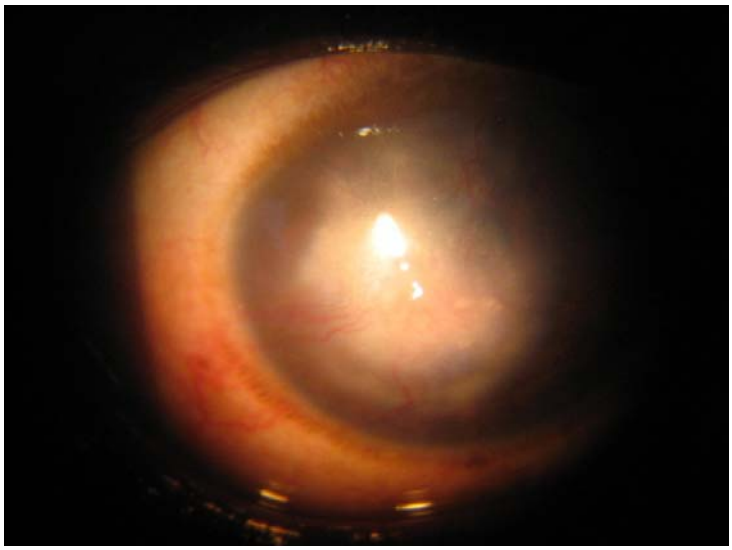


3 Months Post-Injection

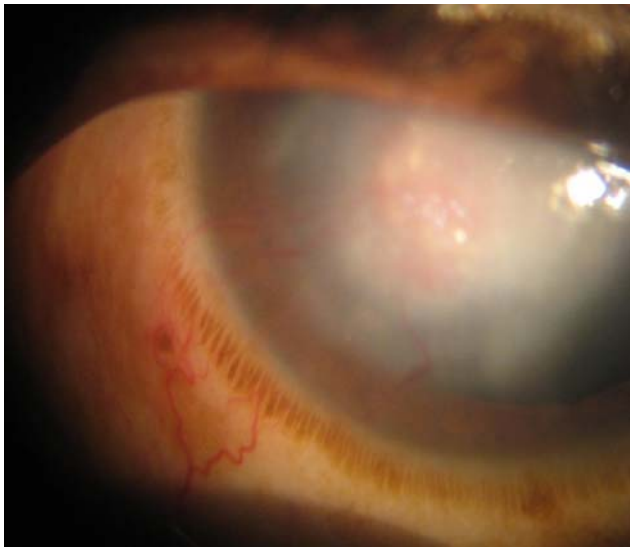


b. PARTIAL REGRESSION

CASE-1
Post Traumatic Leucomatous Opacity

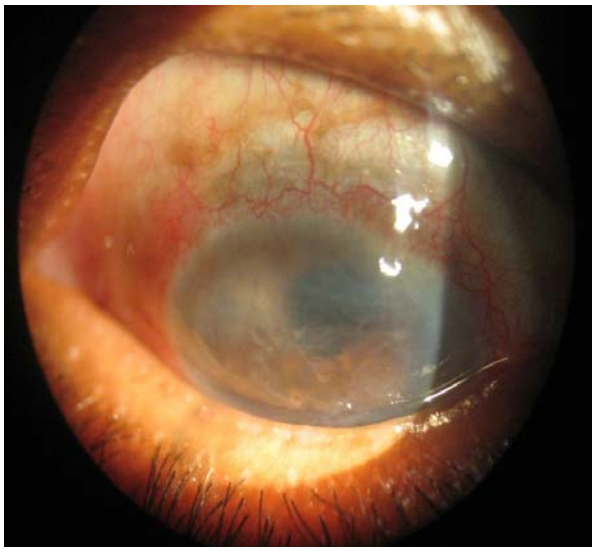


3 months pot injection

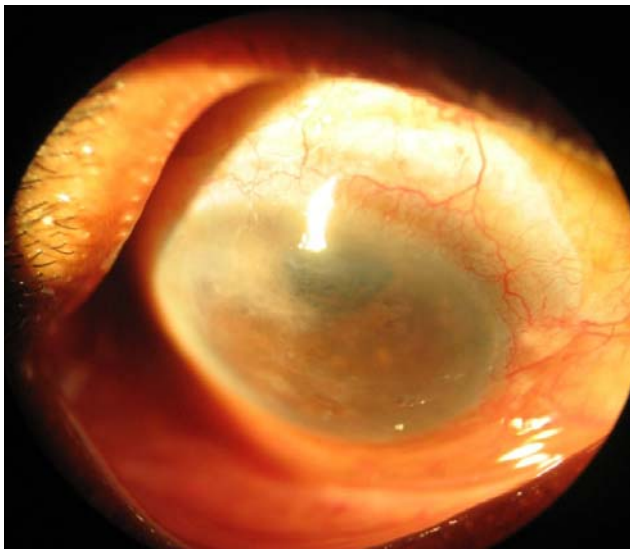


CASE-2

Pseudophakic Bullous Keratopathy
Pre-Injection

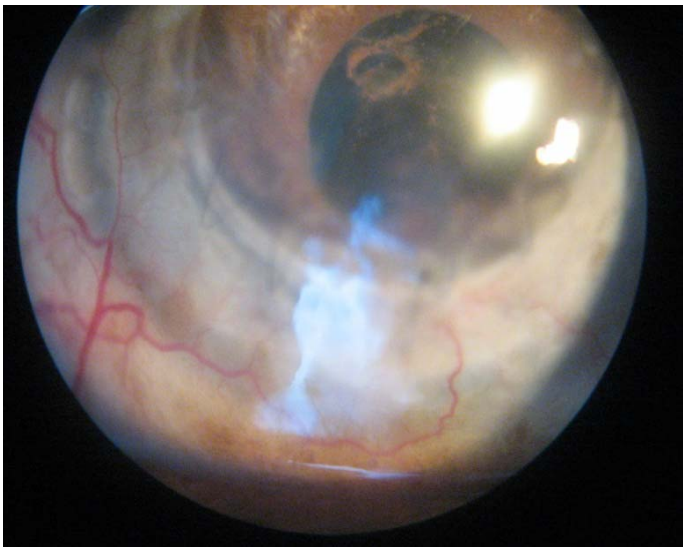


3 Months post-injection

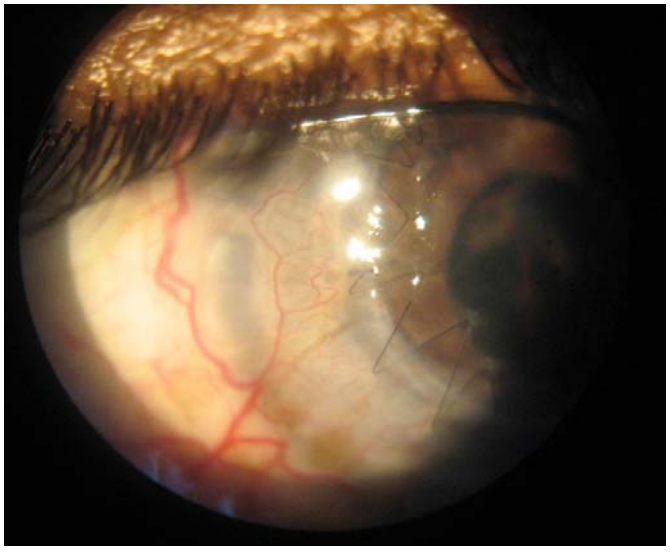


c. NO REGRESSION

2 Months Post-Injection



6 Months Post-Injection



B .SAFETY OF BEVACIZUMAB

- Subconjunctival injection of Bevacizumb was well tolerated by all these twenty patients.

- During this 6 months follow-up period none of the eyes developed any complication that could be related to subconjunctival injection of Bevacizumab.

We found no corneal epithelial defect, corneal ulcer, corneal oedema, conjunctival necrosis, anterior uveitis, or infectious endophthalmitis related to subconjunctival injection in this study. However, the safety of bevacizumab on corneal cells is still an issue that needs to be considered. Yoeruek et al .demonstrated that bevacizumab is not toxic to corneal cells of human origin in vitro at doses normally used for treatment of corneal NV (up to 5.0 mg/ml), which is 20-fold higher than the dose used for intravitreous application.

Kim et al. showed that topical application of bevacizumab causes spontaneous loss of corneal epithelial integrity and progression of stromal thinning. However, our study and others seem to support the conclusion that the subconjunctival delivery method is a good option for

inhibiting corneal NV. The delivery method is easy and simple to perform and leads to minimal related complications.

C. COUTCOME OF OKP

- ❖ Out of these 20 patients, 12 underwent optical keratoplasty and there was no immediate rejection during this 6 months follow-up period.

DISCUSSION

Corneal neovascularization remains a significant risk factor for corneal transplantation and subsequent graft failure after transplantation. The Collaborative corneal transplantation study identified, in addition to several other factors, the extent of stromal vessel (quadrants) involvement as a strong risk factor for corneal graft failure[14].

Corneal neovascularization also affects millions of long term contact lens wearers, many of whom are forced to do reduce or discontinue contact lens use so that regression of corneal neovascularization may occur.

Various medications and surgical therapies have been attempted to reduce corneal angiogenesis including corticosteroids, non-steroidal anti inflammatory drugs, laser photocoagulation and needle diathermy. Many of these therapies are associated with adverse effects and risks, and most of these therapies have demonstrated limited success. Furthermore none of these treatments specifically target the inhibition of VEGF[3&15].

Anti-VEGF therapy, used to reduce corneal angiogenesis, has recently granted interest based on the successful results demonstrated with intravitreal bevacizumab. Preliminary in vivo animal data have shown the potential effectiveness of bevacizumab in treating corneal neovascularization.

Amino et al reported a potent suppression of corneal neovascularization after stromal implantation of anti-VEGF antibodies using a rat model. Ren et al also used a rat model to show the effects of topical bevacizumab (4mg/ml) in decreasing corneal neovascularization when applied twice daily for 1 week[7]. In topically administered bevacizumab there is no complete regression of the new vessels. This may be due to incomplete diffusion & absorption of the drug through the conjunctiva and due to washing out of the topical drug by the tear film & hence inadequate access to the ocular surface. Studies have proven that subconjunctival bevacizumab of 10mg is not superior to 5mg in regression of new vessels[3].

This is a 6 month prospective, non randomized clinical study to investigate the safety and efficacy of subcojunctival bevacizumab for the treatment of corneal neovascularization.

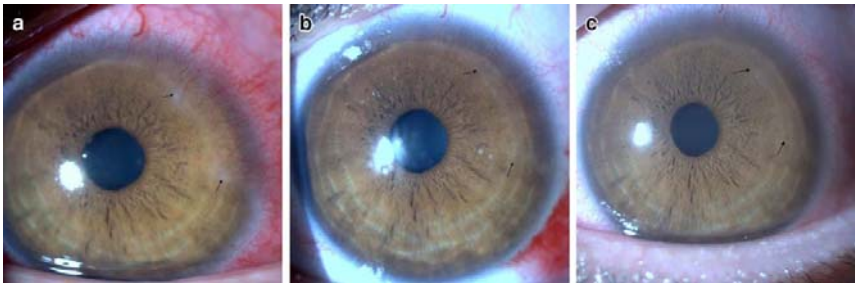
In this study an obvious reduction in established corneal neovascularization occurred to a different degree in each patient, and subconjunctival bevacizumab was well tolerated by all these patients. Out of 20 patients in 50% 0f patients complete regression of the corneal new vessels noted at the end of 6th month. Whereas in 30% of patients only partially reduction noted i.e. reduction in calibre of vessels only, the number of new vessels was constant. In 20% of patients both number of new vessels and the calibre was constant. All these 4 patients had large calibered vessels and the duration of the disease process was also long.

This variable response may be because of the chronicity, extent of corneal neovascularization, amount of scarring, disease process, formulation and route of administration of the drug [15&16].

Successful reduction of corneal neovascularization with subconjunctival anti-VEGF therapy could play an important role in improving graft survival in patients who have pre-existing corneal neovascularization (NV) or NV of the peripheral cornea that develops after penetrating keratoplasty. The reduction or elimination of corneal NV could therefore allow for corneal transplantation[17] in those patients who were previously considered high risk and had contraindication to surgery.

REVIEW OF LITERATURE

The off-label use of topical as well as subconjunctival bevacizumab has also been considered as a new treatment modality for corneal NV.



Corneal neovascularization (NV) due to aqueous-deficient dry eye with filamentary keratitis (a). It was rapidly resolved, one week after subconjunctival bevacizumab injection (b). No relapse was seen at the end of three months of follow-up (c)

Corneal neovascularization (NV) due to corneal graft failure (a). Minor branches of the corneal neovascularization appear to be rapidly resolved one week after subconjunctival bevacizumab injection (b), while the major branch did not markedly regress within the two months of follow-up (c)

Briefly, a dramatic regression of corneal NV in all eyes was confirmed by slit-lamp biomicroscopy within just a week after injection and no relapse was seen within the follow-up period of 2–3 months. In this study they used 2.5 mg (0.1 ml) of bevacizumab solution for corneal NV in patients. The results suggested that this dosage may be enough for corneal NV and could be repeated if necessary. Although some portion of bevacizumab might have been passed into the systemic circulation via subconjunctival vessels, a sufficient amount seemed to be maintained to lessen the corneal NV[7].

Our study findings provide evidence that anti-VEFG therapy could potentially offer a safer and effective alternative to conventional therapies in treating corneal neovascularization without potential adverse effects. The initial impressive short term response and the high tolerance to subconjunctival bevacizumab therapy offer encouraging results for the potential role of subconjunctival anti-VEGF therapy in treating corneal diseases associated with corneal NV.

CONCLUSION

- Subconjunctival injection of Bevacizumab can be used safely and effectively for corneal neovascularization resulting from different types of disorders.

- It may provide an additional strategy in improving success of corneal grafts in these patients.

- This is a short term study. However long-term follow-up is necessary to determine whether repeat injections are necessary.

PROFOMA

Case No;

Name

Age

Sex

Occupation

Op No

Cornea Clinic No

Phone No

Address

Chief Complaints

Pain, watering, photophobia Eye;

Defective vision

Opacity

Duration

Past History

History of injury

History of previous surgeries

Diabetes

Hypertension – No of years

On what medications

Associated with ischemic heart disease

Cerebrovascular insults

Anticoagulant intake

History of smoking

Systemic Examination

PR; BP; RBS;

Ocular Examination

Visual acuity before and after dilatation

Intraocular pressure

Slit Lamp Examination

Conjunctiva

Cornea – extent of corneal opacity & neovascularization drawn with colour pens, photographs taken.

Anterior chamber

Iris

Pupil

Lens

Dilatation and fundus by 90D with Slit Lamp and Indirect Ophthalmoscopy

DIAGNOSIS;

Treatment history

Under aseptic precautions, under topical anaesthesia injection bevacizumab 2.5mg (0.1ml) injected in _____ quadrant.

OUTCOME OF TREATMENT;

	present	**absent**
Decrease in no of vessels	+	-
Decrease in calibre of vessels	+	-
Signs of rejection	+	-
Side effects	+	-

CONSENT;

KEY TO MASTER CHART

M	**-**	**Male**
F	**-**	**Female**
C.C NO	**-**	**Cornea Clinic Number**
LE	**-**	**Left Eye**
RE	**-**	**Right Eye**
PBK	**-**	**Pseudophakic Bullous Keratopathy**
FCU	**-**	**Fungal Corneal Ulcer**
LEUCP	**-**	**Leucomatous Opacity Post Chicken Pox**
PO TR	**-**	**Post Trauma**
HYD LEUC	**-**	**Post Hydrops with Leucomatous Opacity**
REGRAFT	**-**	**Previously failed graft for repeat OKP**
OKP	**-**	**Optical Keratoplasty**
INJCN	**-**	**Bevacizumab Injection given**
PO	**-**	**Prior to OKP**
DO	**-**	**During OKP**
AO	**-**	**After OKP**
WL	**-**	**Weeks Later**
ML	**-**	**Months Later**

N↓	**-**	**Reduction in the number of the vessels**
C↓	**-**	**Reduction in the Calibre of vessel**
REJCN	**-**	**Signs of graft Rejection**
S.E	**-**	**Side Effects**
+	**-**	**Present**
-	**-**	**Absent**

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LIST OF SURGERIES PERFORMED

S. No	Name	Age	Sex	IP No	Diagnosis	Surgery
1	Shantha	65	F	693412	BE-MC	RE-ECCE with PCIOL
2	Karpagam	70	F	683675	BE-MC	RE-ECCE with PCIOL
3	Sundaram	60	M	727681	RE-IMC LE-MC	RE-ECCE with PCIOL
4	Dhanalakshmi	55	F	732612	RE-IMC LE-MC	LE-ECCE with PCIOL
5	Govindan	65	M	757345	RE –PSEUD/LE MC	LE-ECCE with PCIOL
6	Nagarajan	56	M	785492	RE-MC	RE-ECCE with PCIOL
7	Rahimbee	55	F	790053	LE-IMC	LE-ECCE with PCIOL
8	Kannamma	70	F	820789	RE-MC	RE-SICS with PCIOL
9	Shiva	64	M	853412	RE-PSEUD/LE IMC	LE-SICS with PCIOL
10	Sundar	59	M	87720	RE-IMC	RE-SICS with PCIOL
11	Rani	65	F	89558	LE-IMC	LE-SICS with PCIOL
12	Prema	54	F	90567	RE-IMC	RE-SICS with PCIOL

13	Balu	50	M	640091	LE-IMC	LE-SICS with PCIOL
14	Hema	49	F	543781	RE-IMC	RE-PHACO with PCIOL
15	Sukumar	50	M	549834	LE-IMC	LE-PHACO with PCIOL
16	Ramachandran	65	M	65479	RE-IMC	RE-PHACO with PCIOL
17	Yesudass	69	M	98712	RE-POST OP ENDOPH	INTRAVITREAL ANTIBIOTICS
18	Saroja	76	F	25378	RE-PANOPH	RE-EVISCERATION
19	Alagiri	60	M	65498	LE-CDC	LE-DCT
20	Subramni	55	M	76594	RE-CDC	RE-DCT
21	Anandh	62	M	69831	RE-FUNGAL CORNEAL ULCER	RE-TKP
22	Visalam	60	F	72254	RE PERFORATED ULCER	RE-TKP
23	Sumathi	45	F	76583	RE-CORNEAL ULCER	RE-TKP
24	Ramachandran	75	M	35471	LE-PTERYGIUM	RE-EXCISION withAMG
25	Rajamani	55	M	37659		
26	Lakshmi	30	F	275498	RE-ABSOLUTE GLAUCOMA	RE-TRAB

27	Rajendran	44	M	65890	LE-CDC	LE-DCR
28	Meenakshi	45	F	78659	RE-CIN	EXCISION
29	Kaliammal	68	F	28779	RE-EXP.KERATITIS	RE-LATERAL TARSORRAPHY
30	Mary	57	F	79463	RE- CORNEAL ULCER	RE-TKP

MASTER CHART

S. No.	NAME	AGE	SEX	C.C NO	DIAGNOSIS	INJCN	OKP	2 WEEKS POST INJECTION				1 MONTH POST INJECTION				3 MONTHS POST INJECTION				6 MONTHS POST INJECTION			
								N↓	C↓	REJCN	S.E	N↓	C↓	REJCN	S.E	N↓	C↓	REJCN	S.E	N↓	C↓	REJCN	S.E
1	Appayee	70	M	917	RE-PBK	PO	2WL	+	+	-	-	+	+	-	-	+	+	-	-	-	-	-	-
2	Murugan	30	M_	1540	LE-FCU	PO	5ML	+	+		-	+	+		-	+	+	-	-	-	-	-	-
3	Selvaraj	55	M	1095	LE-LEUCP	DO		+	+	-	-	+	+	-	-	+	+	-	-	-	-	-	-
4	Lakshmi	22	F	3657	RE-HYD LEUC	AO		+	+	-	-	+	+	-	-	+	+	-	-	-	-	-	-
5	Mohammadh	62	M	4283	RE-PBK	PO	3ML	+	+		-	+	+		-	+	+	-	-	-	-	-	-
6	Elumalai	50	M	4074	RE-REGRAFT	PO	5ML	+	+		-	+	+		-	+	+		-	-	-	-	-
7	Vasantha	48	F	803	RE-PBK			+	+		-	+	+		-	+	+		-	-	-		-
8	Hussein	58	M	3427	RE-PBK	PO	1ML	+	+		-	+	+	-	-	+	+	-	-	-	-	-	-
9	Muniyandi	35	M	1611	RE-PO TR	PO	1ML	+	+	-	-	+	+	-	-	+	+	-	-	-	-	-	-
10	Allma	68	F	2361	LE-REGRAFT	AO		+	+	-	-	+	+	-	-	+	+	-	-	-	-	-	-
11	Surendarsing	32	M	560	LE-LEUCP	PO	3ML	-	+		-	-	+		-	-	+	-	-	-	-	-	-
12	Babu	46	M	718	LE-TEGRAFT	PO	3ML	-	-		-	-	-		-	-	-	-	-	-	-	-	-
13	Shankar	30	M	296	RE-PO TR	PO		-	+		-	-	+	-		-	+	-		-	-		-
14	Sunder	25	M	415	RE-FCU	PO		-	+		-	-	+		-	-	+	-		-	-		-
15	Hari	69	M	1290	LE-PBK	PO	2ML	-	-		-	-	-		-	-	-	-	-	-	-	-	-
16	Patchaiyappan	50	M	1536	RE-LEUCP	PO		-	+		-	-	+		-	-	+		-	-	-		-
17	Srinivasan	47	M	1772	LE-FCU	PO		-	-		-	-	-		-	-	-		-	-	-		-
18	Dilipkumar	25	M	2837	RE-FCU	PO		-	+		-	-	+		-	-	+		-	-	-		-
19	Akila	15	F	2165	LE-FCU	PO		-	+		-	-	+		-	-	+			-	-		-
20		65	M	1559	RE-PBK	PO		-	-		-	-	-		-	-	-		-	-	-		-