A SINGLE-BLINDED RANDOMISED CONTROLLED TRIAL ON THE EFFICACY OF ADJUNCTIVE COLLAGEN CROSS-LINKING IN HEALING OF SUPPURATIVE CORNEAL ULCERS

DISSERTATION SUBMITTED AS PART OF FULFILLMENT FOR THE MS BRANCH III (OPHTHALMOLOGY) EXAMINATION DEGREE EXAMINATION OF THE TAMIL NADU DR.MGR MEDICAL UNIVERSITY, TO BE HELD IN MAY 2020

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BONAFIDE CERTIFICATE

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INTRODUCTION

The cornea is the clear transparent dome shaped anterior-most part of the eyeball that serves as the major refracting surface for focusing of images on the retina.

Infectious keratitis, or suppurative corneal ulcer, is characterized by a corneal epithelial defect with underlying stromal inflammation and destruction caused by multiplying organisms and their toxins. Associated uveal tissue and anterior chamber inflammation also occur.

A suppurative corneal ulcer is an ocular emergency as it is a potentially sight threatening condition. The commonest etiological agents are bacteria, fungi, virus and protozoa (acanthamoeba). Treatment of a corneal ulcer is challenging since due to the avascularity of the cornea, systemic antibiotics are generally less effective. The standard treatment for bacterial and fungal keratitis is broad-spectrum antibiotic and antifungal drops respectively, preferably after a microbiological assessment to determine the type of organism present, and its sensitivity has been done.(1)

In recent times, there has been an increase in antibiotic resistance of organisms that cause corneal ulcers. (1) With the increase in resistance of microorganisms to antibiotic treatment, newer modalities of treatment need to be sought. Additionally,

microbiological investigations are not always possible due to financial constraints, or are inconclusive of the causative microorganism, which makes treatment more difficult.

Collagen cross-linking (CXL), a procedure routinely used for control of progression of keratoconus, has been found to have beneficial effects on many types of corneal ulcers. An observational pilot study in our institution in 2013-2014, demonstrated a beneficial effect of CXL in suppurative corneal ulcers(2)

This single blinded RCT has been designed based on the results of that study to further investigate this result.

AIM

To determine the benefit of adjunctive collagen cross linking (CXL) in reduction of the "Time to healing" of suppurative corneal ulcers

SECONDARY OBJECTIVES

a) To determine any difference in treatment failure rate (rates of perforation/keratoplasty/evisceration) of corneal ulcers treated with CXL as compared to the control group.

b) To assess the effect of risk factors (size of ulcer/diabetic status/type of organism)

in outcome of corneal ulcer treatment with CXL compared to controls

REVIEW OF LITERATURE

INTRODUCTION

Infectious keratitis, or corneal ulcers, is a leading cause of blindness in developing countries like India and are more prevalent especially in conditions of poor hygiene(3),(4). In addition to the almost 10 times larger load of infective corneal ulcers (3), compared to Western countries, there is also a much larger proportion of fungal ulcers. Unfortunately, the availability of topical medication for fungal ulcers is very limited as compared to antibiotics for bacterial ulcers(5).

Another challenge in the management of corneal ulcers in these regions is the high costs involved with microbiological procedures of culture and sensitivity determination. This has fuelled the practice of using empirical and intensive long-term multidrug treatment for corneal ulcers.(5) The inevitable outcome of this is the increase in multidrug resistance of organisms causing corneal ulcer, associated with relentless worsening of the corneal ulcer, thus increasing the morbidity. This has become a major public health concern.(3),(4),(6)

The high cost of microbiological procedures to determine effective medication as well as the frequency of drug resistance has led to a search for an alternative or adjunctive approach to therapy for microbial keratitis, which can be used where advanced

facilities for microbiological studies are not available, as well as in cases of multidrug resistant keratitis.

Corneal Collagen Crosslinking (CXL) is a procedure that may potentially be such an alternative/adjunctive modality, providing a generalized approach in the management of various types of corneal ulcers in developing countries(7). There have been several case reports of successful management of suppurative corneal ulcers unresponsive to conventional antimicrobial therapy.(8),(7),(9),(10),(11),

THE CORNEA

The anterior dome-shaped part of the eye is formed by the cornea. While protecting the inner contents from the environment, it also serves in providing 65 to 70 % of the eyes refractive power(1).

The Cornea consists of 6 layers

1) outer epithelium,

2) Bowman's layer

3) stroma

4) Dua‘s layer

5) Descemet’s membrane

6) inner endothelium.

The corneal epithelium is the first mechanical barrier to environmental pathogens. It also plays a critical role in the air-tear film interface, which is important for the refractive power of the eye.(2)

The Bowman’s layer is a pseudo basement membrane about 15 microns thick and if injured, does not regenerate, but heals with scarring.

The stroma of the cornea constitutes the bulk of the tissue, and accounts for 90 % of the thickness.(1). Only 2-3 % of the corneal stroma is made up of cellular components (i.e. keratocytes). Greater than 70 % of the dry weight of the cornea is constituted by collagen fibrils. The unique molecular shape, the highly organized lattice arrangement as well as the very regular and evenly spaced fine collagen fibrils blocks the forward scatter of light and contributes to the transparency as well as the mechanical strength of the cornea.

Beneath the stroma lies Dua's layer- a recently described , well defined, acellular layer that is acellular exists in the prescemets cornea. This was discovered by Harman Preet Dua using the big bubble technique. Its discovery has helped in better understanding of corneal biomechanics and posterior corneal surgery. It has also helped in understanding pathology of posterior cornea such acute hydrops, descemetocele and pre- descemets dystrophy.(14)

Beneath the Dua's layer is the Descemet's layer which gives support to the single layer of cells in the endothelium.

TRANSPARENCY OF THE CORNEA

The relative dehydrated state, the avascularity, the uniform refractive index of the corneal layers and uniform spacing of the collagen fibrils in the stroma, all contribute to the transparency of the cornea.

The highly complex levels in which the collagen fibrils are arranged as well as the uniform size of collagen fibrils is very important for corneal transparency.

The mean distance between collagen fibers in stroma and the mean diameter of these fibres are relatively homogeneous and is less than half the wavelength of visible light (400–700 nm). This is the reason why the scattering that is produced by an incident ray of light on each collagen fiber is cancelled, thereby making the cornea transparent. The diameter of the collagen fibrils is approximately 25–35 nm. These fibrils are arranged from limbus to limbus parallel to each other and in layers called lamellae (200–250 nm thick). (15)

NORMAL STRUCTURAL CHANGES IN THE CORNEA WITH AGING

There are various physiological processes that happen with aging. There is a decrease in the the hydration stability and amount of glycosaminoglycan in the cornea. Along with this, there is increase in the glycation and non- enzymatic crosslinking of the collagen (16). This is a normal process that occurs in aging and is because of advanced glycation products that accumulate in the stroma. This type of increased cross linking results in added strength to the cornea, and has been shown to benefit patients with keratoconus.(17) This is why the natural progression of untreated keratoconus beyond the age of 35 - 40yrs is very minimal.

CORNEAL ULCER

A corneal ulcer is a lesion with superficial loss of tissue i.e. the epithelium with infiltration and inflammation of the underlying stroma. Depending on the etiology, these can be sterile or infective.

The different causative organisms for microbial keratitis can be fungal, bacterial, viral or parasitic.

This study specifically relates to bacterial and fungal keratitis.

RISK FACTORS

There are several risk factors that contribute to the development of microbial keratitis such as contact lens use, trauma and foreign bodies, previous ocular surgery and

exposure to contaminated water. Fungal ulcers are common after trauma with vegetative and soiled particles.

Deranged local host defences such as following ocular chemical injury, in uncontrolled diabetes, neurotrophic disease, with lid or lash malposition, dry eye, or deficiency of stem cells as well as previous herpetic disease all constitute risk factors. Topical corticosteroids and topical anaesthetics can also hamper the local defence mechanisms.

EPIDEMIOLOGY

According to WHO, there are about 36 million blind people worldwide.(18). In India there are approximately 6.8 million people estimated to have vision less than 6/60 in atleast one eye.(4). Every year it is estimated that 25,000 to 30, 00 corneal blindness cases are added to the total burden in the country.

Also 90 % of corneal blindness that occur due to corneal ulceration and ocular trauma, leading to corneal blindness occurs in developing countries.

The prevalence of blindess due to corneal pathology is reported to be 0.45% i.e. approximately 5.4 million people.(4)

STANDARD TREATMENT FOR CORNEAL ULCERS

BACTERIAL ULCERS

Bacterial ulcers are rapidly progressive and need emergency treatment. Clinical diagnosis by an experienced practitioner and microbiological report are essential for treatment of any corneal ulcer. Emperical treatment with either fluoroquinolone monotherapy(19),(20),(21) or combination therapy with fortified drops(cefazolin +tobramycin /gentamicin) can be used(22). Empirical therapy is important while waiting for the culture reports. The frequency depends on the severity. For most ulcers one hourly drops is sufficient , but half hourly can be given if it is very severe. In severe ulcers a loading dose of a drop every 5 minutes can be used. Based on the response, the frequency of the drops is tapered.

Fortified aminoglycosides that are used as eye drops are gentamicin and tobramycin. These have an excellent Gram-negative coverage, and are effective against *staphylococci* and some *streptococci* but not against *pneumococci* . They are however epitheliotoxic. Fortification is done by adding 80 mg/2 ml of antibiotic injection to 5 ml of commercially available antibiotic eye drops (0.3%) to get a concentration of 1.35%.

Cefazolin is the most commonly used cephalosporin. It has good coverage for non-penicillinase producing Gram-positive bacteria. It is prepared by adding 5 ml of sterile water for injection, to Injection Cefazolin 250 mg. The drops are refrigerated and should be discarded if they discolor to yellow or after a week, whichever is earlier.

Monotherapy with fluoroquinolones is still being debated. Practitioners now prefer to use higher generation drugs such as Gatifloxacin and Moxifloxacin due to development of antibiotic resistance.(1)

Signs of improvement of corneal ulcers are:(1)

1. Stabilization and no progression of infiltrate
2. Reduced activity at infiltrate margins / blunting of ulcer edges
3. Reduction in adjacent stromal inflammatory reaction and anterior chamber inflammation
4. Resolution of infiltration and progressive healing of epithelial defect.

FUNGAL ULCERS

Treatment of fungal ulcers can be more challenging than bacterial ulcers. It is difficult to diagnose and treat fungal ulcers and sometimes advanced ulcers may resemble bacterial ulcers. Moreover they require more time for growth in cvculture media and appropriate sensitivity testing is limited.

First choice for treatment of filamentous fungal keratitis is Natamycin 5%. Therapeutic scraping helps to remove slough and reduce load of infection. It also helps in drug penetration. Drops are used every half to one hourly initialy. Response to treatment in fungal infections is very slow. The drops are tapered according to clinical response. It may take about 4 to 8 weeks for the complete resolution of the ulcer.

Amphotericin B is the first choice against yeasts. But it is not as effective as Natamycin for filamentous fungi. In refractory cases, Amphotericin B (0.15%) drops can be considered alone or in combination with Natamycin; however, their penetration through an intact epithelium is less than Natamycin.

Intracameral (5-10 µg) Amphotericin B has also been used successfully in patients refractory to topical and oral antifungals. Amphotericin B is available as a 50-mg injection. Drops are prepared by adding 10 ml water for injection to the vial to get a stock solution (5 mg/ml). 3.5 ml of water for injection is added to 1.5 ml of this stock

solution to get 5 ml of 0.15% drops, which can be dispensed. The drops should be refrigerated and should not be exposed to light.

The azoles and fluocytosine are generally employed as alternative agents for advanced ulcers or for ulcers not responding to polyenes. Topically applied, the imidazoles have poor corneal penetration, and so they are more effective in treating superficial infections especially due to *Aspergillus* . Topical Itraconazole 1%, Clotrimazole 1%, Ketoconazole 2%, Econazole 2%, and Miconazole 1% have also been used for fungal keratitis. Voriconazole has the broadest spectrum of the azole antifungals and has good intraocular penetration after oral administration. Voriconazole is a new, promising therapy for fungal keratitis that is refractory to standard antifungal agents. (23),(24)

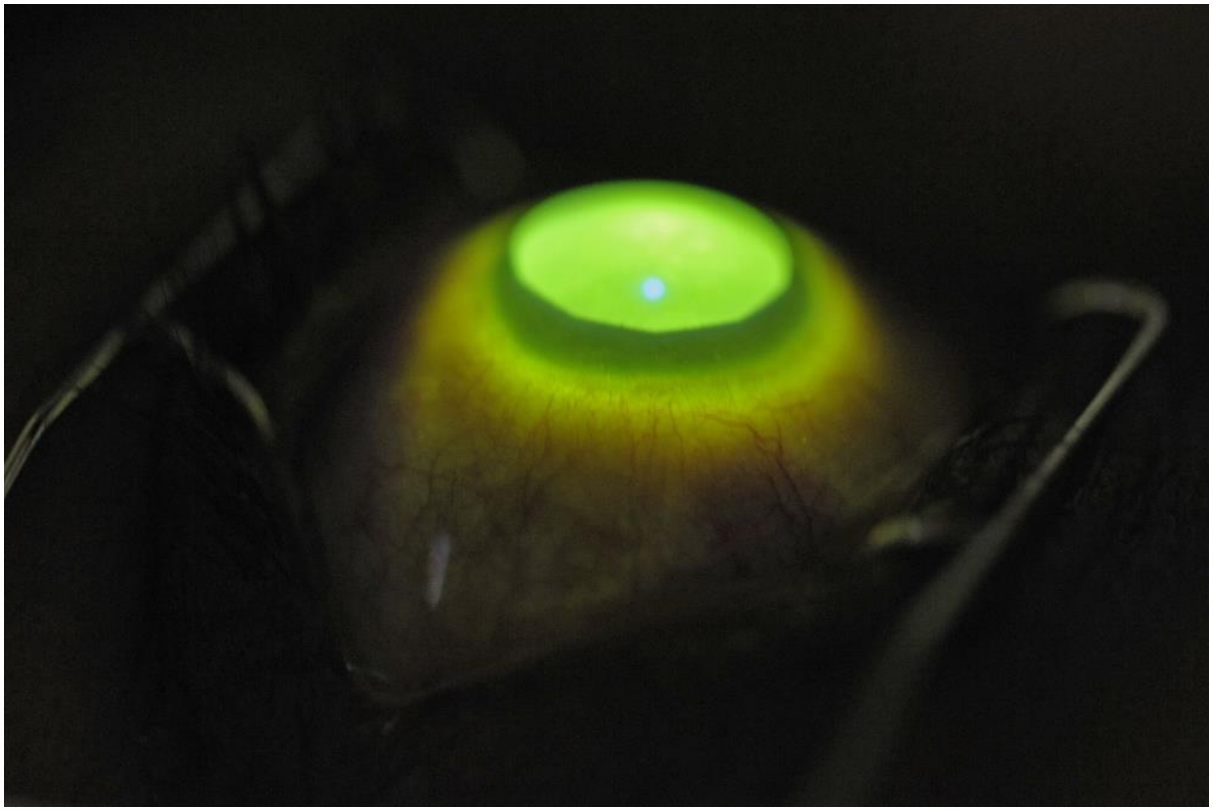
COLLAGEN CROSS LINKING:

Cross-linking is a natural process that occurs with aging in the cornea. Corneal collagen cross-linking (CXL) was first introduced in 1997 by Spoerl et al as a treatment modality for ectatic cornea(25). It is a non-invasive technique being successfully used worldwide to halt the progression of keratoconus.[13]

According to the standard Dresden protocol UV-A treatment is applied over central 9mm zone of cornea using an irradiance of 3.0 mW/cm2 at a duration of 30 min, thereby delivering a fluence of about 5.4 J/cm2.(26)

The procedure involves shinning a UV light of a known intensity and diameter on the cornea for 30 minutes utilizing a Riboflavin-based photosensitizing dye solution as drops on the cornea during the procedure to augment the effect of UV light on the superficial cornea(27).

This therapy has proven safe for the cornea and is even used in children, with no long-term detrimental side-effects.(27),(28) Enhanced cross-linking produces a stiffening effect, with no loss of corneal clarity, thus halting the progressive degeneration that occurs in keratoconus patients.(27),(28),(29)



RATIONALE FOR EFFICACY OF CXL IN TREATMENT OF CORNEAL ULCERS:

Studies have demonstrated that corneas treated with CXL have increased resistance against enzymatic digestion and corneal melting.(30),(31) Additionally, the free oxygen radicals released during crosslinking have an antimicrobial effect as they interfere with integrity of the cell membrane.[18] Furthermore, UV light and Riboflavin as a photosensitizer has been used successfully in the decontamination of blood products prior to transfusion.(32),(33)

Bacterial corneal ulcers results in corneal melting due to increased activity of collagenase (both tissue and microbial origin). This can lead to vision threatening condition like perforation and loss of eye.

Kohlhaas M et al and Spoerl et al in the university of Dresden studied 20 enucleated porcine eyes that under went crosslinking -and they found that the treated cornea had significant stiffening in the anterior 200 microns as compared to the posterior 1/3 rd and control corneas.(29)

Cross linking helps in in increasing the biochemical and mechanical stability of the cornea. Till recently cross linking was a treatment modality for melting processes and disorders that weaken the cornea. It is being used for treatment of Keratoconus, pellucid marginal degeneration and keratectasia following LASIK surgery.(34)

RIBOFLAVIN

It was in the year1932 that Paul Gyrogy discovered vitamin B2, now known as riboflavin. In 1934 Richard Kuhn isolated the substance called flavin and later it was also noticed to have a ribitol molecule thus being termed riboflavin. It is used in medicine mainly for two purposes – photosensitization of blood products and for corneal collagen cross linking.

Activation of riboflavin UV-A light can selectively damage DNA and RNA of pathogens and thereby reduce replication of bacteria, protozoa, and viruses in blood products.(35) Riboflavin can also activate leukocytes under controlled conditions, without significant compromise of the efficacy of blood products. Riboflavin absorbs UV-A radiation and acts as a photosensitizer, which causes release of reactive oxygen species. During cross linking, UV-A light exposure on riboflavin causes formation of radicals that cause cross linking. Riboflavin drops contains dextran, which helps in maintaining the osmolarity It also prevents corneal swelling and soaking.

Photoactivated riboflavin causes damage to DNA and RNA of microorganisms through oxidative processes, thereby causing lesions in chromosomal strands(36). The riboflavin intercalates in between the bases of DNA and RNA and oxidates the nucleic acids. This antimicrobicidal property of riboflavin, which is being used in transfusion medicine (37)brings us to the hypothesis that it may also benefit it treatment of infectious keratitis(33).

LABORATORY STUDIES

Toxicity of direct UV – A light and endothelial damage

Direct exposure of the eye to UV radiation can cause damage to the lens cornea and the retina. It has been found to cause cataract in the lens, photokeratitis in the cornea and photochemical toxicity of the retina(34).

Wollensak et al found in his study that the damage threshold with combination of UV-A and riboflavin was 10 times lower than UV-A alone (0.35 vs. 4 mW/cm). But, because of the shielding effect of riboflavin, the damage threshold to the endothelium is smaller by at least a factor of 2. (38)

In this study, the right eye of 34 new Zealand rabbits were cross linked. The endothelial cells were then assessed using the TUNEL technique. They concluded that combined riboflavin–UVA treatment is safe for the endothelium if the dose is smaller than the endothelial cytotoxic dose of 0.65 J/cm^2. In human corneas, this dose is only reached the deep stroma in corneas that are thinner than 400 µm. (39)

<u>Resistance to corneal melting in cross linked corneas</u>

Kohlhaas et al in 2006 studied the stiffening of cornea in 20 eyes of rabbit that underwent collagen crosslinking. In their study they found that the anterior 200 microns of the corneas that underwent crosslinking had significant stiffening as compared to the control group and the posterior part of the cross linked corneas. (26)

Spoerl et al in 2004 studied the resistance of cross linked corneas to the enzymatic degradation by pepsin and trypsin . They found that it took half the time for enzymatic degradation in the eyes that did not undergo cross linking as compared to the cross linked corneas, i.e. the crosslinked corneas were more resistant to enzymatic degeneration.(31)

In a study done by Makdoumi etal in 7 human eyes, they reported that cross linking halted the progression of corneal melting in infective corneal ulcers and also helped in complete epithelialization.(40)

Antimicrobial properties of photosensitized riboflavin

Ruane et al in 2004 studied the reduction of pathogens in platelet concentrate with help of a device that uses UV-A light and compound riboflavin. In his study, he found that there was significant difference in the viral (porcine parvo virus) and the bacterial (staph epidermidis and E. coli) load. However, it was also concluded that there was no significant difference in the clinical significance of using treated versus untreated platelet concentrate. (41)

Photo-activated riboflavin can induce chromosomal damage in microbes by binding to the DNA and RNA.(41),(37)

Additionally, direct irradiation by UV light has microbicidal and sporicidal effect. (42)

In vitro studies on antimicrobial properties

Martin et al in 2008 did a study on bacterial and fungal isolates. He studied the effect of UV-A and riboflavin on two group of microbes. He used Kirby Baur discs with various bacterial and fungal isolates. They found, inhibition of growth of both drug sensitive and drug resistant organisms. However, there was no effect against the growth of candida albicans.(43),(44)

A study by Schrier et al in 2009 on staph aureus, MRSA and pseudomonas aerogenosa showed that agar plates exposed to both UV-A and riboflavin showed bacterial death as compared to Agar plates that was exposed only to riboflavin or only to UV A light. This suggested that the combination of riboflavin and UV-A light is bactericidal.(45)

In 2010 Makdoumi et al did a study on psudomonas aeruginosa, staph epidermidis and staph aureus and showed that combination of riboflavin and

UV-A had a better effect on eradication of bacteria as compared to that of UV light alone.(46)

Sauer et al in 2010 studied the effect of UV-A and riboflavin on Fungal isolates from patients with severe fungal corneal ulcers. Candida albicans, fusarium and aspergillus fumigatus was studied. The conclusion was that amphotericin B may diffuse easily following crosslinking. The effectiveness of UV-A / riboflavin was increased following previous treatment with Amphotericin.(47)

Kashiwabuchi et al in 2011 studied the efficacy of long wave UV-A with riboflavin on fungal colonies of candida albicans and fusarium . But the study concluded no beneficial effect.(48)

A study on growth of acanthamoeba in the presence of UV- A and riboflavin was conducted by Makdoumi et al in 2013. It was found that exposure to riboflavin did inhibit the growth of acanthamoeba but the addition of riboflavin did not show any change.(49)

In 2016 Makdoumi et al did a similar study on antibacterial action but with using the settings of PACK -CXL. It was found to effective against both antibiotic resistant and non resistant strains.(50)

IN VIVO STUDIES

The following is the available literature on the small case series and few case reports on the benefit of cross linking in infective keratitis in humans.

Iseli et al in 2008 conducted a study on 5 patients with corneal ulcer not improving with topical antibiotics. Each patient underwent one session of crosslinking. Out of the 5 patients, 4 patients showed regression of corneal melt and infiltrate size while one patient with fusarium ulcer worsened and had to undergo therapeutic keratoplasty in 4 weeks. The other cultured microbes were 2 non tubercular mycobacterium, acremonium and mycobacterium chelonae. The authors concluded that crosslinking was a promising modality of treatment for infective keratitis that does not respond to broad spectrum antibiotic treatment.(30)

In 2009 a case report of a 78 year old women (known diabetic) with e coli corneal ulcer was reported by Micelli Ferrari et al .The ulcer had not responded to topical antimicrobial agents. One session of cross linking was done following which there was

significant improvement of symptoms and development scarring. After a month there was healing of the ulcer and resolution of corneal oedema. (9)

Moren et al in 2010 reported a case of a 25 year old female who developed severe unilateral infective keratitis, she was a contact lens user. As the conventional treatment for 1 month showed no benefit one session of cross linking was tried. Following the procedure within a few days there was symptomatic improvement with re-epithelialization. In 2 months the ulcer had completely healed and 9 month BCVA was 20/30.(51)

In 2010 a case series of 7 eyes of 6 patients (3 patients were contact lens users) with severe infectious keratitis was conducted by Makdoumi et al. The duration of symptoms ranged between 0 to 7 days. All cases had associated corneal melting. The symptoms of patient improved in all but 1 eye. There was further prevention of corneal melting and hypopyon, which was present in 2 eyes, had regressed completely. They concluded that collagen cross linking could be an effective treatment modality in infectious keratitis.(40)

Sorkhabhi et al in 2013 conducted a non randomized case series on collagen cross linking in resistant corneal ulcers. Ten patients of which nine were caused by

staphylococcus and one aspergillus ulcer was studied. Within 48 hours there was improvement of symptoms in 8 patients - such as epiphora, pain and photophobia. Within 3 months all cases had complete epithelialization and arrest of corneal melting. In the other two cases one patient underwent therapeutic keratoplasty and the other patient underwent evisceration. Those two cases had deep stromal ulcers. It was suggested that deep ulcers (>300 micron) may not be sufficiently acted upon by riboflavin and UV-A light.(8)

In 2014 we completed an observational cohort pilot study to determine if collagen crosslinking showed the same promise as other authors had reported, in patients with our profile of corneal ulcers. The study comprised of a prospective cohort (cases; Exposure: CXL), and a retrospective/historical cohort (controls) (REF: Cornea 2018. Nov;37(11):1376-1380) I have put this in the intro also.

The patients in the prospective cohort consisted of patients who presented during the period April – October 2014, who fulfilled the study criteria. They were all treated with standard antimicrobial medical therapy following microbiological analysis. These patients underwent additional CXL as per the Dresden Protocol (3mW/cm for 30 min, dose 5.4 J/cm). Antimicrobial therapy was continued after the procedure.

The retrospective or historical cohort (controls) consisted of age and sex matched patients who had a similar profile of suppurative corneal ulcers as the prospective cohort, satisfying the inclusion and exclusion criteria who had been treated in our institution prior to the start of this study. In order to increase the power of the study we took at least 2 controls per case. Medical details were extracted from their medical records.

The inclusion criteria for both groups included patients between 18 and 75 years of age, corneal ulcer size 2-6 mm and a positive smear or culture for bacteria or fungus, who where willing for in-patient care. The patients in the prospective cohort should, additionally, have been able and willing to give an informed consent.

Exclusion criteria included **s**uspected viral keratitis or proven Acanthamoeba keratitis, corneal thinning >50%, patients with history of previous CXL and patients who were unable to understand or unwilling to consent.

Cross-linking has previously been found to be non-contributory and even detrimental in Viral and acanthamoeba ulcers, as UV light has been found to re- activate Herpes keratitis, and case-series with acanthamoeba ulcers has not given good results.(52),(53)

The time to healing in both cohorts were then compared.

We found that in patients where CXL was performed healed about 20 days faster than the retrospective cohort of patients (p=0.6), which, though not reaching statistical significance, is particularly clinically significant. However, diabetes mellitus was found to be a significant confounder in that study.

Most of the literature available till 2014 consisted of case reports and case series. Since then, two comparative trials (one randomized) have been published. (54,55)

In 2014 Said et al studied 40 patients with advanced infectious keratitis and corneal melt (organisms: bacterial, fungal, acanthamoeba, mixed).(54) In this non-randomized comparative trial, nineteen patients underwent treatment with conventional medications only, while 21 patients underwent additional crosslinking.

The average time until healing was 39.76 ± 18.22 days in the CXL group and 46.05 ± 27.44 days in the control group of patients of up (P = 0.68). The corneal ulceration's width and length was significantly bigger in the CXL group (P = 0.004 and P = 0.007). The complication rate was 21% in the control group, whereas there was no incidence

of corneal perforation in the CXL group. The authors concluded that there was no difference in the “time to healing” in both groups.

The second trial was a randomized clinical trial published in 2015 by Bamdad et al on 32 patients (16 per arm) with moderate bacterial keratitis (2-6mm size:2/3 corneal thickness involved).(55)

The interventional group underwent one sitting of CXL prior to starting antimicrobial therapy, while the second arm was randomized to only conventional therapy.

They found a healing time of 17.2 +/- 4.1 days in the CXL group and 24.7 +/- 5.5 days in the control group ($p< 0.001$). There was one perforation (failure) in the CXL group and 2 in the control group. The authors here concluded that there was a significant reduction in the “time to healing” as well as perforation rates in the CXL group.

The in vitro efficacy of cross linking on acanthamoeba keratitis was studied by Del Buey et al . four study groups with two different strains of acanthamoeba with UV-A +/- riboflavin was studied. The results of the study showed that a single dose i.e. 30 or 60 minutes of crosslinking did not eradicate the two different strains that were examined.

Based on the literature available, and our own observational study, we believe there is clinical equipoise regarding the efficacy of CXL in treatment of suppurative corneal ulcers. Hence we now want to use the data obtained from our pilot study to perform a single-blinded randomized controlled clinical trial to establish whether this modality of treatment can be added to our armamentarium for treatment of corneal ulcers.

In our pilot study, 10 out of 11 patients required 3 - 4 sittings of CXL at 48 hr intervals. Hence we have decided to standardize our treatment protocol for this RCT to 3 sessions of CXL/Sham CXL at 48 hr intervals for all our subjects.

MATERIALS AND METHODS

Study Design:

Single-Blinded, Randomized, Controlled Clinical Trial conducted at Department of Ophthalmology, Christian Medical College, Schell Campus, Vellore

The study had two arms

Interventional group:

Patients with infective corneal ulcer who satisfy the inclusion and exclusion criteria on standard medical therapy randomized to adjunctive COLLAGEN CROSS-LINKING (CXL)

Comparative group:

Patients with infective corneal ulcer who satisfy the inclusion and exclusion criteria on standard medical therapy randomized to SHAM CXL

a. Inclusion Criteria:

1) Adults greater than 18 years of age

2) Corneal ulcer size of 2mm to 6mm

3) Ulcer infiltrate depth upto 2/3 of the corneal thickness

4) Smear and/or culture positive for fungus or bacteria

5) Patients who are willing for inpatient care

b. Exclusion Criteria:

1) Suspected viral keratitis

2) Suspected acanthamoeba keratitis

3) Corneal thinning greater than 50% on clinical assessment at presentation

4) Any pre-existing corneal pathology

5) History of previous collagen cross-linking

6) Patients who are unable or unwilling to give consent

Outcome:

Success:

Healing of the ulcer; End point – no evidence of active infiltrate with complete closure of epithelial defect

Failure:

Non-healing:

1. Loss of the integrity of eyeball (perforation of cornea)

2. Emergency therapeutic corneal transplantation

3. Evisceration (removal of eyeball contents due to uncontrolled infection)

4. Progressive thinning of ulcer more than 50% during the course of the trial.

Exposure: Corneal Collagen Crosslinking (CXL)

Sample size calculation:

Sample size calculation was based on the observational pilot study from our department in 2014. In that study, the mean "time to healing" was 49 (SD:37) days in the Control (retrospective) group, while this was 23 (SD:14) days in the prospective intervention group. In order to show this difference statistically significant with alpha and beta errors at 5% and 20% respectively we need to study 18 subjects in each arm.

Adding 15% for drop outs, we decided to take **21 patients per arm**

Method of randomization:

Block randomization was done with blocks of 2 and 4. The proportion of blocks was 40% and 60% respectively. These were mixed in order to avoid prediction. The randomization scheme was done using SAS software.

Method of allocation concealment: Sealed Envelope provided by the statistician

Blinding and masking:

The primary investigator performed the CXL/Sham CXL based on the randomization. The secondary investigators (thesis guide and co-guides) were blinded to the treatment given, and made the clinical assessments of healing or non-healing.

METHODOLOGY

Patients with suppurative corneal ulcers were admitted after routine microbiological analysis (scraping for smear and culture), and assessed on a daily basis to determine response to treatment. All patients who fit the inclusion and exclusion criteria and who were randomized to CXL, underwent UV-A/riboflavin cross-linking (CXL) within 48 hours of admission. CXL was performed in three sessions, with an interval of 48 hours between therapy sessions.

Every morning throughout the admission period, the patients were assessed and graded with respect to

Reduction of pain
Rounding of corneal infiltrates
Reduction of hypopyon
A subjective 'forced gut feeling' of healing

The grading scale was the same as was used in the pilot study and was as follows:

Parameters	Grading		
	-1	0	+1
Reduction of pain			
Rounding of corneal infiltrates			
Reduction of hypopyon			
A subjective 'forced gut feeling' of healing			
Total score			

The "time to healing" (complete closure of the epithelial defect) or non-healing (loss of integrity of the eyeball, emergency corneal transplantation or progressive thinning of the cornea >50%) was determined.

Procedure for Collagen Crosslinking:

Corneal collagen crosslinking was performed under sterile conditions in a room dedicated for the procedure. Under topical anesthesia, loose epithelium and the

debris was wiped away. Riboflavin (K-Link - riboflavin/dextran solution 0.1%) was topically applied to the cornea as drops, for a period of 30 minutes at interval of 3 minutes. UV irradiation was then performed with a commercially available apparatus (Appasamy UV Irradiator) using a wavelength of 365 nm and irradiance of $3m/W/cm^2$ for a further 30 minutes with topical administration of riboflavin continued during this period at 3 minutes interval.

This treatment was repeated at 2-day intervals for 3 sittings. Before each sitting, corneal thinning was assessed to make sure that removal from the study due to corneal thinning exceeding 50% was not indicated.

APPASAMY UV A LIGHT SOURCE

UV meter and protection goggles

Calibration of UV light

Timer and UV intensity control

Procedure for Sham Crosslinking

This was also performed under sterile conditions in the same room dedicated for CXL. Under topical anesthesia, loose epithelium and the debris was wiped away.

Artificial tear drops were topically administered to the cornea for a period of 30 minutes at interval of 3 minutes as drops. Following this, blue light from a torch covered with blue cellofane paper was applied on the eye for 30 mins while artificial tear drops were continued at 3 minute intervals.

This was repeated at 2 day intervals for 3 sittings.

SHAM CROSS LINKING WITH BLUE LIGHT

STUDY ALGORITHM/FLOW CHART

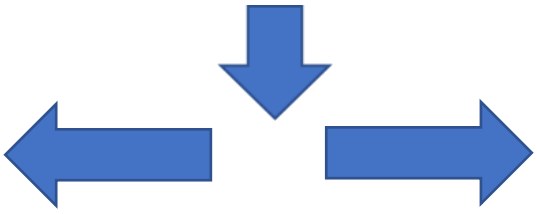
Patient seen in the department of ophthalmology (OPD)

Microbiological scraping for smear and culture taken

Antibiotic/Antifungal therapy started as per existing protocol

If eligible (after smear/culture reports come) as per study criteria, consent taken

Patient block randomized using SAS software



Collagen cross linking performed within 48 hrs of admission after smear/culture reports are available

patient exposed to blue light (torch with a blue filter)

l

Collagen cross linking repeated 3 times with 48 hrs interval

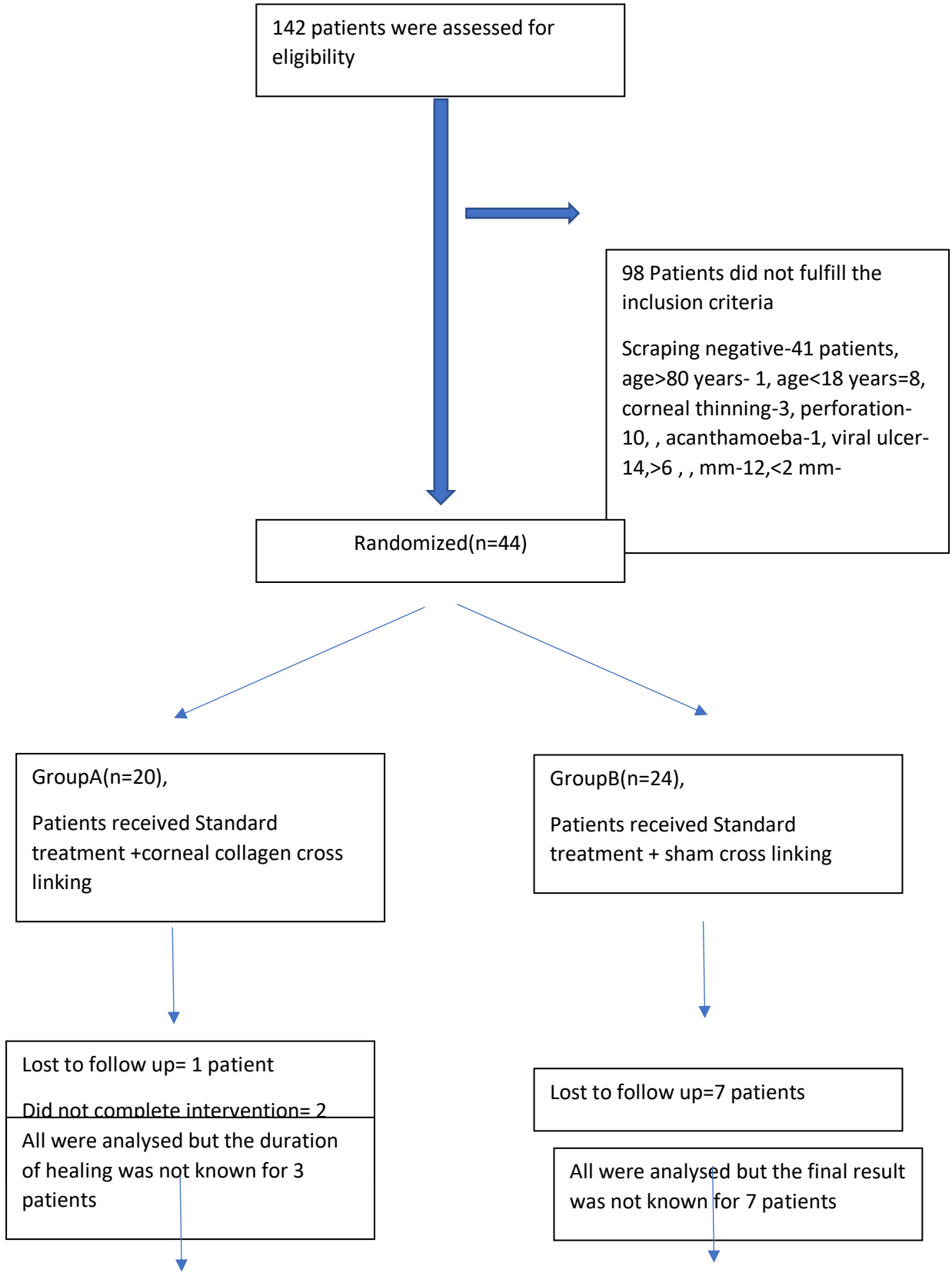
Repeated 3 times at 48 hrs interval

Time taken for epithelial defect to completely heal/non-healing (loss of eyeball integrity/ emergency corneal transplantation /progressive thinning >50%) recorded

Statistical Analysis

RESULTS

Flow Diagram



DEMOGRAPHY OF THE STUDY GROUP

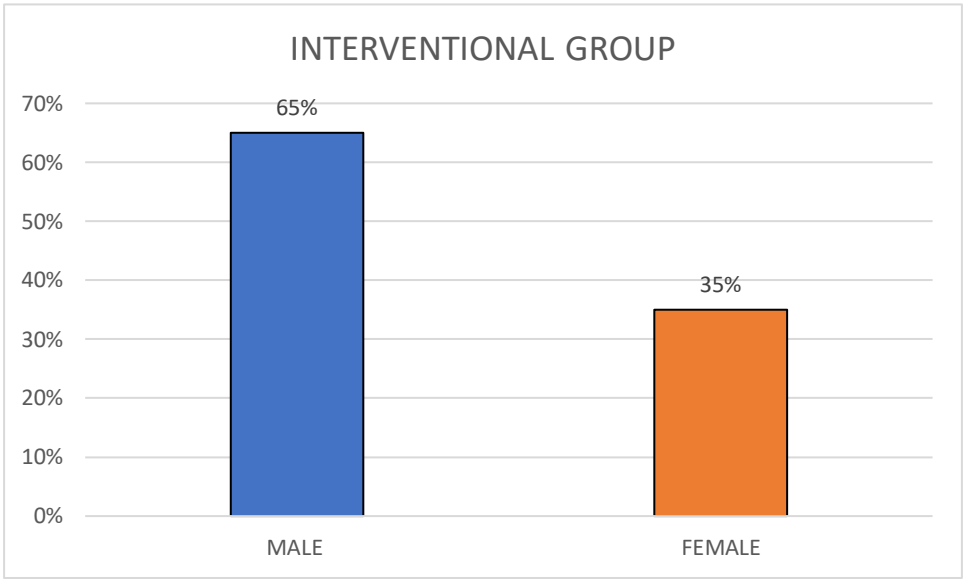
a. Gender Profile Of Patients In The Study Group

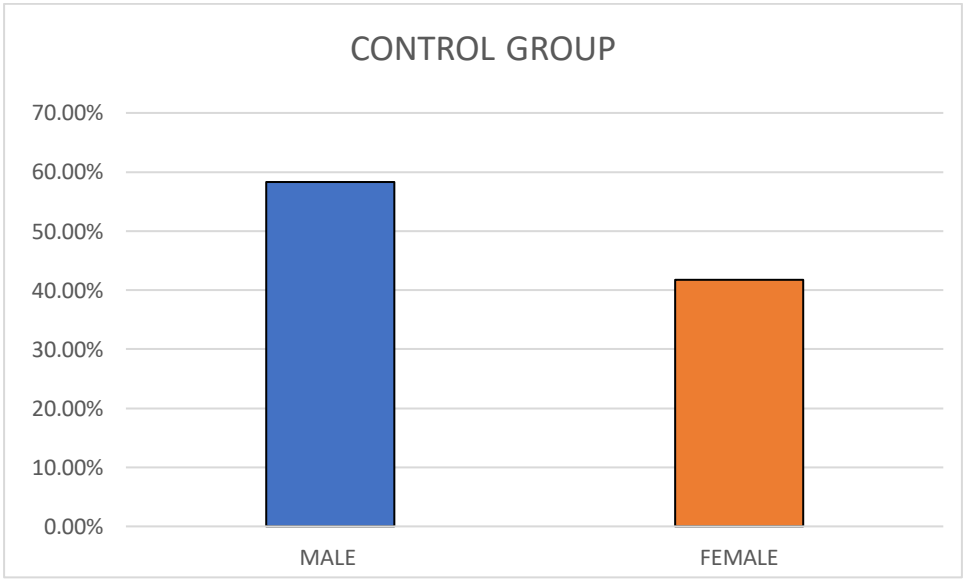
Table 1 : gender profile of the patients in intervention group and control group

GENDER	INTERVENTION GROUP	CONTROL GROUP
MALE	13(65%)	14(58.30%)
FEMALE	7(35%)	10(41.70%)

In both groups there were more males as compared to females.

The following chart shows the pictorial representation of the gender distribution comparing each group.





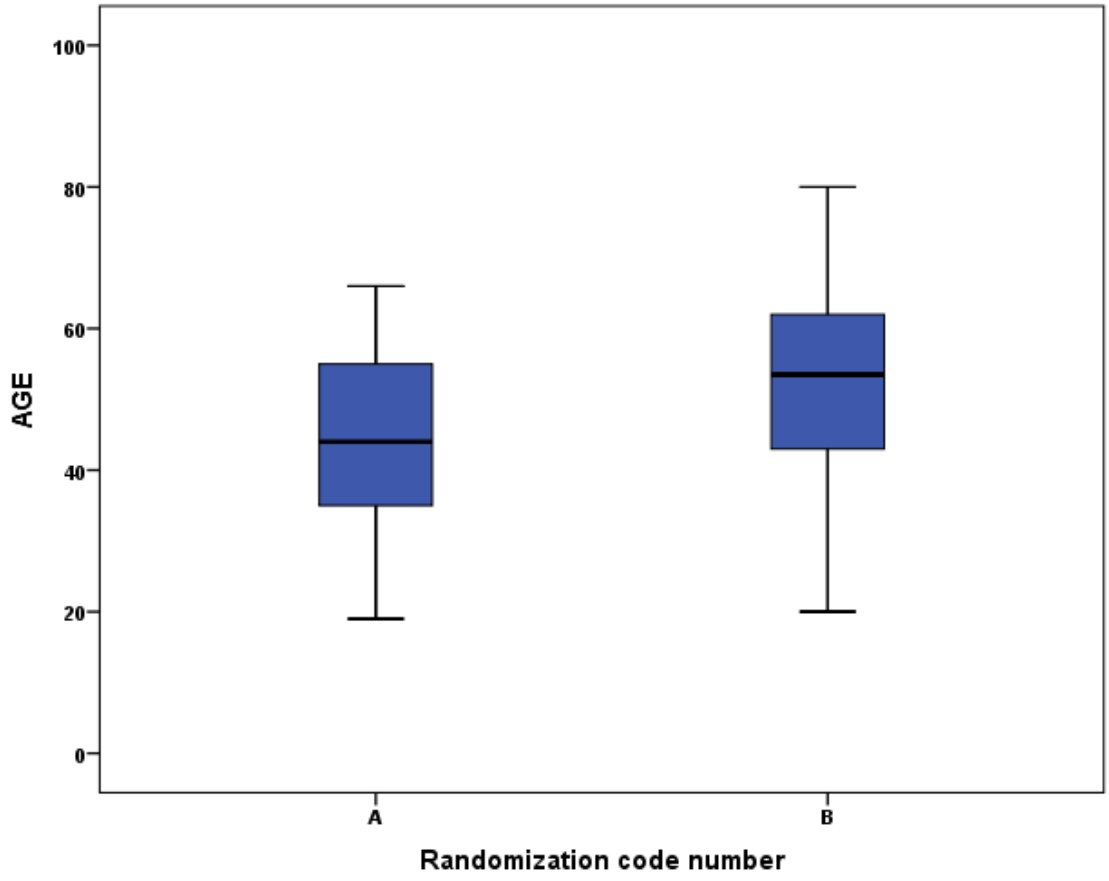
b. AGE DISTRIBUTION

INTERVENTIONAL GROUP

	Minimum	maximum	mean
Age	19	66	45

CONTROL GROUP

	Minimum	maximum	Mean
age	20	80	52



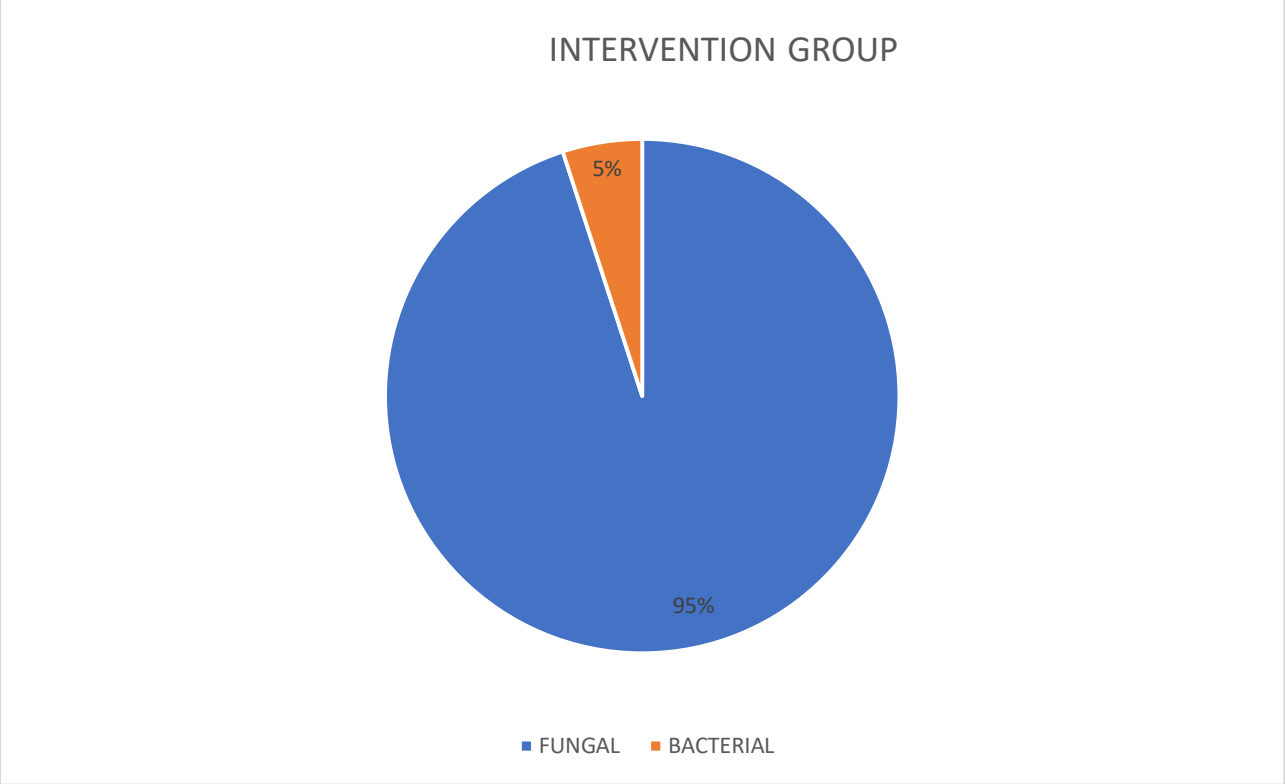
A-INTERVENTION GROUP	B- CONTROL GROUP

2. CAUSATIVE ORGANISMS

a. TYPE OF ULCER-IN EACH GROUPS

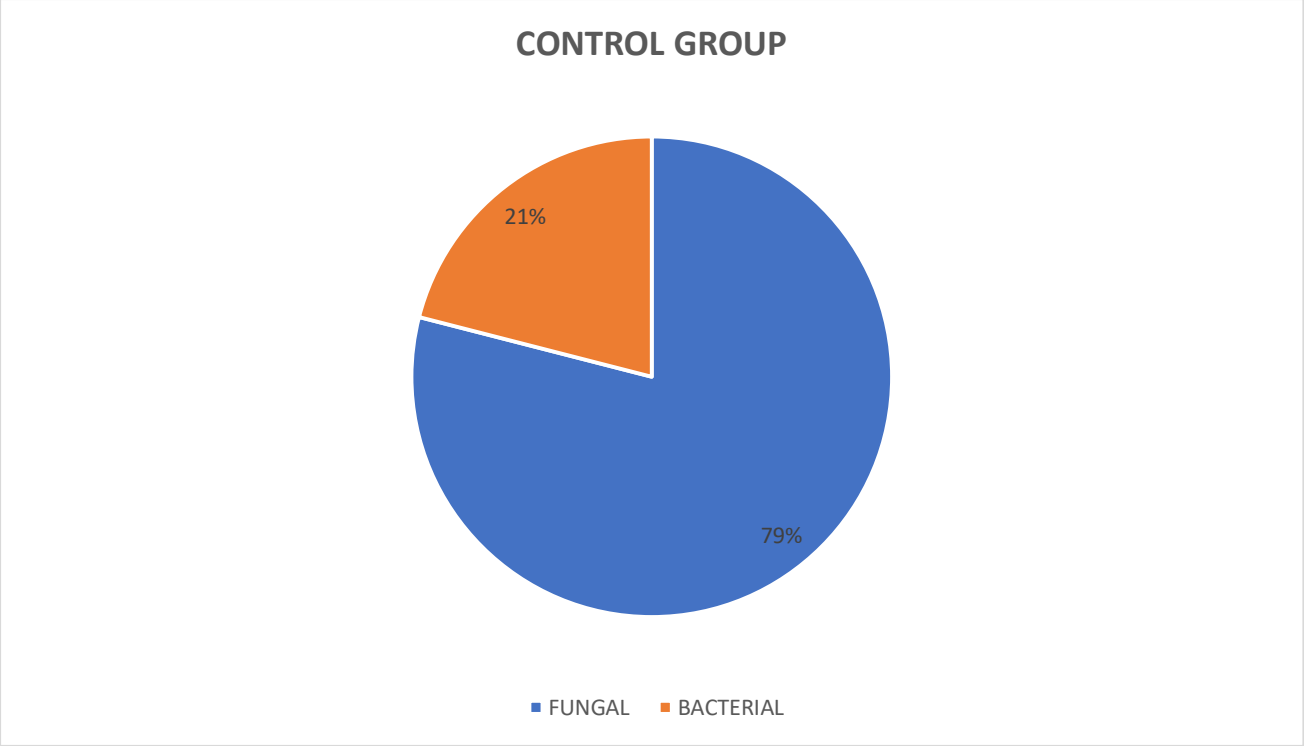
Type of ulcer- intervention group

TYPE	INTERVENTION GROUP
FUNGAL	19
BACTERIAL	1



Type of ulcer – Control group

TYPE	CONTROL GROUP
FUNGAL	19
BACTERIAL	5



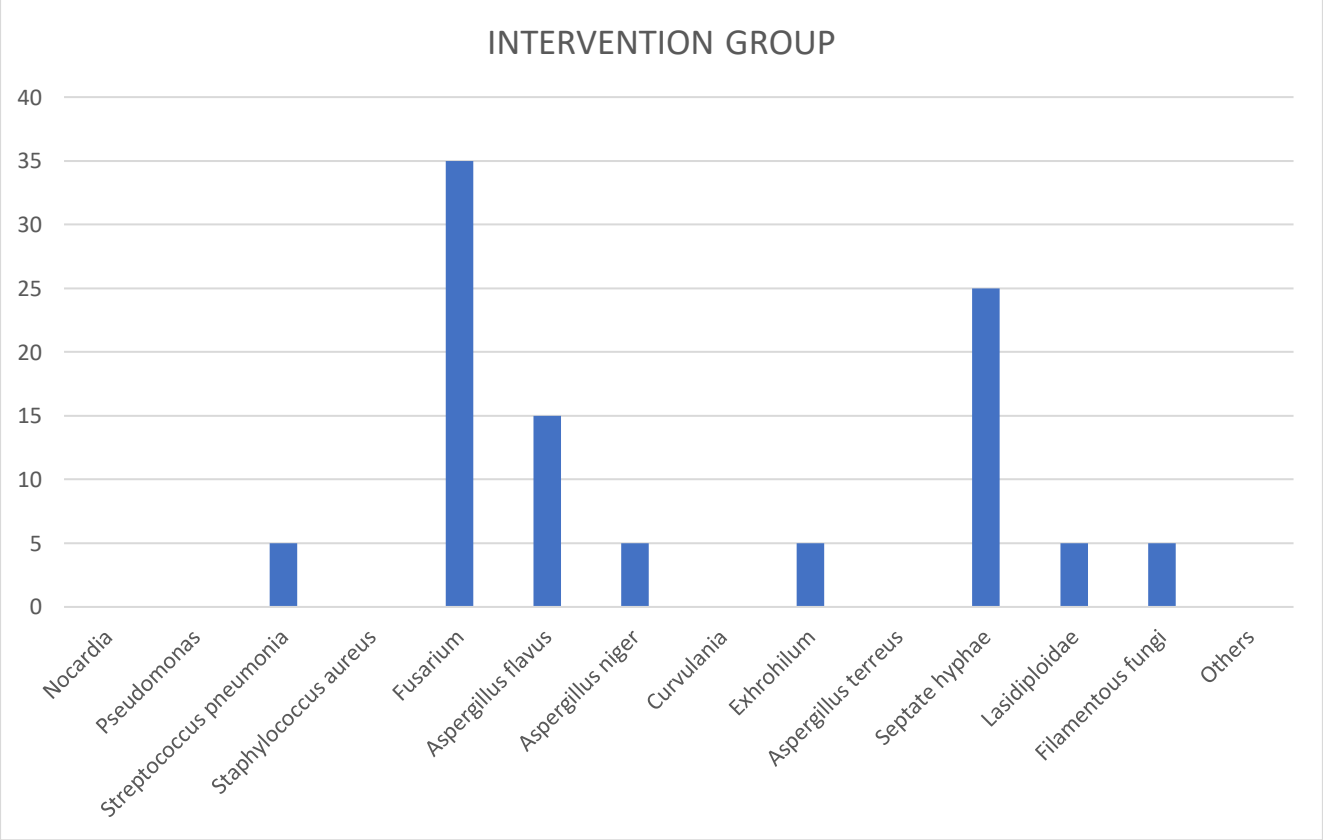
In both groups, fungal ulcers far outnumbered the bacterial ulcers.

However, it can be seen that there were more bacterial ulcers in the control group as as compared to the intervention group.

This can be attributed to the fact that more fungal ulcers than bacterial ulcers are seen in our department; and it was a common randomization for both bacterial and fungal ulcers.

b. Etiologic Profile of Ulcers in the Intervention group

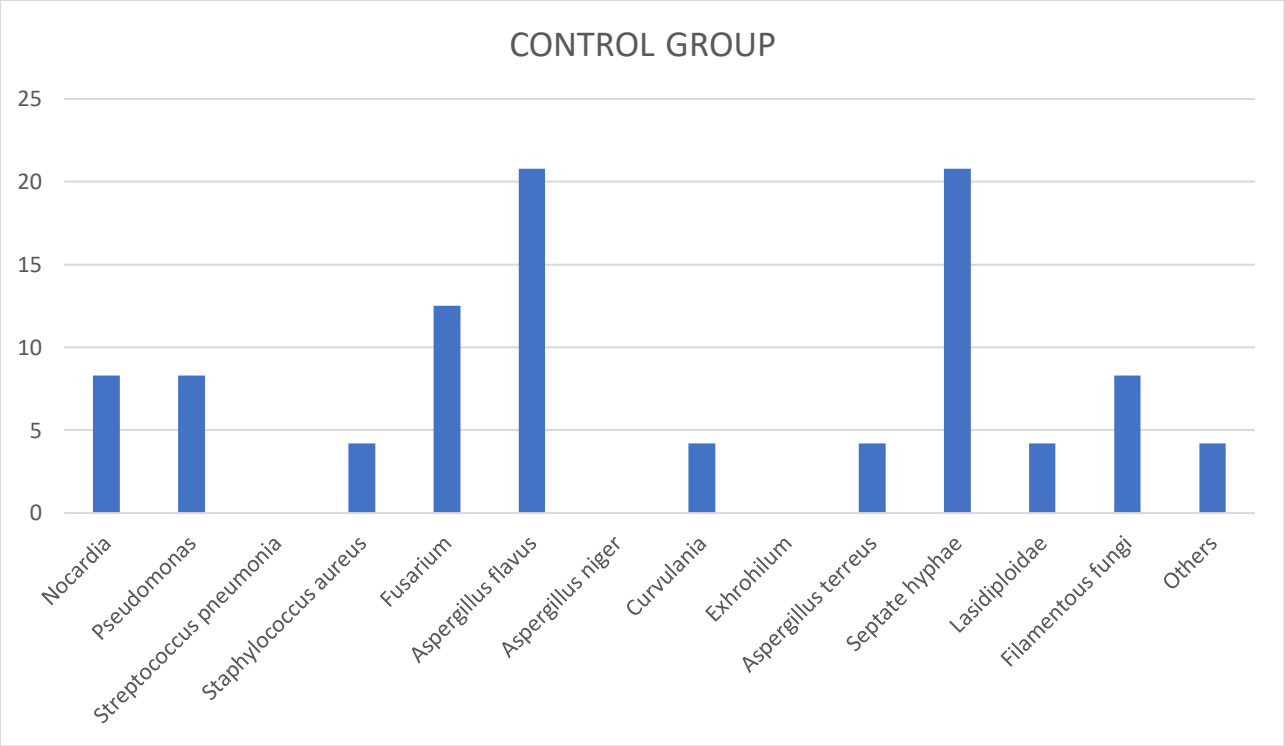
INTERVENTION GROUP



Fusarium ulcers were almost double the number (35 vs 20) as compared to Aspergillus ulcers in the intervention group. This was similar to the results of our pilot study, and is comparable with other data from southern India.

c. Etiologic Profile of Ulcers in the Control group

CONTROL GROUP



Unlike in the Intervention group, Aspergillus ulcers were more as compared to fusarium ulcer in the control group.

3. CHARACTERISTICS OF THE CORNEAL ULCERS

a. Epithelial defect size

INTERVENTIONAL GROUP		
	Epithelial defect size (vertical)	Epithelial defect size (horizontal)
Mean	3.547	3.521
Std. Deviation	1.0746	1.2555
Mean Area of Ulcer	13.4126 mm^2	

CONTROL GROUP		
	Epithelial defect size (vertical)	Epithelial defect size (horizontal)
Mean	3.227	3.195
Std. Deviation	1.0343	1.1206
Mean Area of Ulcer	10.9768mm^2	

The area was found to be 13.4126 and 10.9768 mm square in intervention and control group respectively. There was no statistically significant difference noted between the areas of the two groups(p = 0.317)

c. Hypopyon

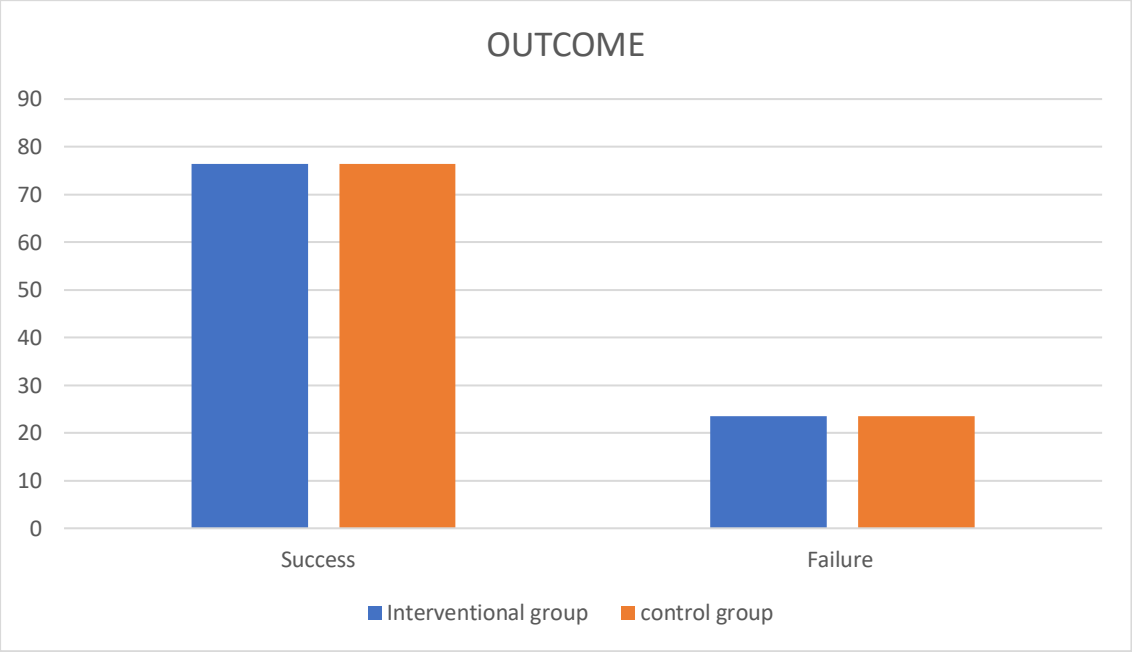
It was found that nine patients in the intervention group and six patients in the control group had Hypopyon. There was no statistical significant difference between the groups (p = 0.337)

4. OUTCOME

Number of patients with successful outcome, and failure of outcome

Outcome	INTERVENTION GROUP	CONTROL GROUP
Success (healed ulcers)	76.47%(13 patients)	76.47%(13 patients)
Failure (corneal perforation, corneal thinning, therapeutic keratoplasty)	23.52%(4 patients)	23.52%(4 patients)

The outcome was exactly the same in the 2 study groups.



5. TIME TO HEALING

The duration of days taken to heal in the intervention group and the control group were analyzed.

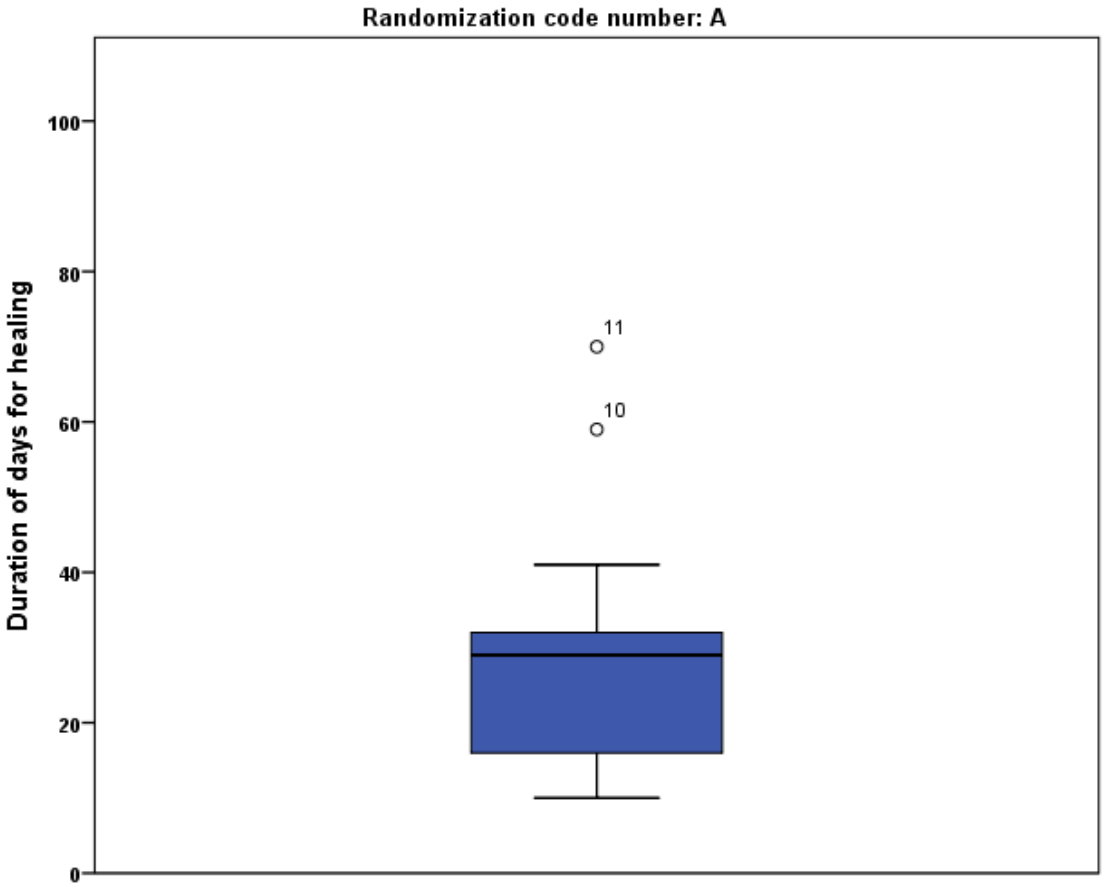
	INTERVENTION GROUP	CONTROL GROUP	difference in mean between groups
Mean(standard deviation in days)	29.85 (17.911)	29.85 (18.907)	0 days

The difference the duration of days required for healing of ulcer in each group was not statistically significant (p = 0.892).

Time to healing in days

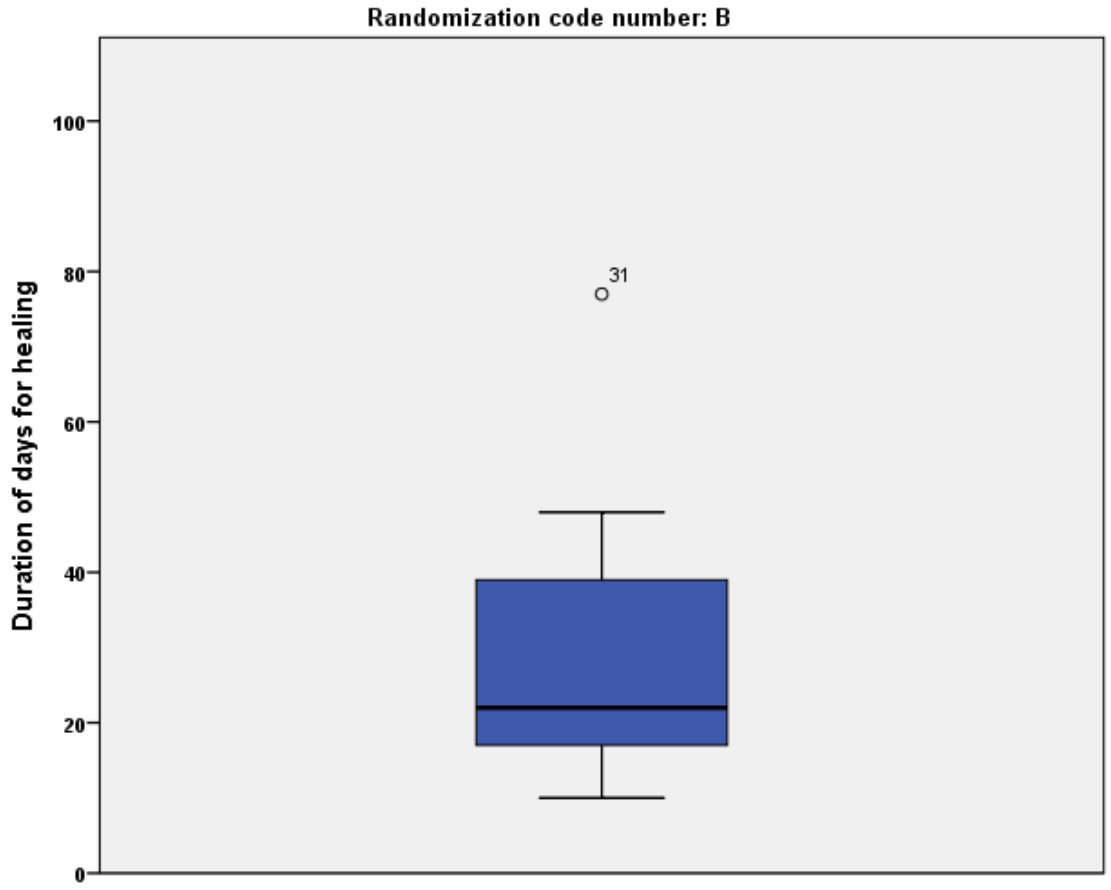
Randomization	Mean±SD	Median(IQR)	P Value
Intervention Group	29.85 ±17.91	29(16,32)	0.892
Control Group	29.85 ±18.907	20(16,37)	

Diabetes			
Yes	37.25±20.27	40(25,50)	0.317
No	27.5±17.68	22(16,32)	



In the intervention group, the median interquartile range was 29.85 days with a range of 12 to 48 days.

The range of days the ulcer took to heal was between 10 days (minimum) to 77 days (maximum) in the Intervention group.



In the control group, the median interquartile range was 29.85 days with a range of 11 days to 49 days.

The range of days the ulcer took to heal in the Control group was between 10 to 77 days.

We analyzed the data again after removing the outliers in each group. One patient in the Intervention group and one patient in the Control group took 77 days and 70 days respectively for the healing of ulcer.

	INTERVENTIONAL GROUP	CONTROL GROUP	difference in mean between groups
Mean(standard deviation in days)	26.5(13.827)	25.92(13.076)	0.58 days less in group B

However, even after removing the outliers it was found that there was no significant difference between the mean Healing time in both groups.

INTERVENTION GROUP : SIZE OF LESION, PRESENCE OF HYPOPYON, AND HEALING TIME

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Group: A			
EPITHSIZ	EPITHSI1	HYPOPYON	DURDAYS
3	3	YES	19
5	5.5	YES	. NIL
3	2	YES	. NIL
4	4	YES	29
5.5	5.5	YES	16
4	4	YES	. NIL
3	2.5	YES	70
4.5	5	YES	12
2	2	YES	. NIL

CONTROL GROUP: SIZE OF LESION, PRESENCE OF HYPOPYON, AND HEALING TIME

Group: B			
EPITHSIZ	EPITHSI1	HYPOPYON	DURDAYS
3.4	3.4	YES	.NIL
3.8	4	YES	35
4.5	3	YES	. NIL
2	2.8	YES	39
4.5	3.7	YES	. NIL
2.7	2.4	YES	. NIL

There was no significant difference note in the duration of days of healing (p value-0.678) nor the size of the ulcer(p value-0.373).

DISCUSSION

This study was undertaken based on our previous pilot study to further investigate whether CXL can reduce the Time to Healing in suppurative corneal ulcers.

Even with intensive treatment with topical antimicrobial (one hourly instillation) and in some cases intra cameral and systemic antifungal it usually takes weeks for healing of the ulcer, also necessitating in-patient care for days or weeks.

Several reasons for the long duration taken for healing of the ulcer exist.

One reason may be the fact that the cornea is avascular with tight junction of epithelial and endothelial cells, thus prohibiting an effective immune response.

However, other reasons are the high cost of the investigations and the requirement of inpatient care, which deters patients from seeking help. Additionally, noncompliance to using the antimicrobial drops or lack of understanding is other factors that can lead to delay in healing.

If the smear report is not confirmatory for any pathogen then the ulcer is treated based on the clinical picture and experience of the treating doctor. In such situation broad

spectrum antibiotics are used and a cocktail combination of antibacterials and antifungals may also commonly be given.

Such use of anti microbials drugs may result in resolution of the ulcer but can give rise to more antibiotic resistant strains. Non healing ulcers and drug resistance is a challenging for the treating ophthalmologist.

Collagen cross linking is a procedure that has been tried out in response to the need for finding therapies that can reduce the duration of the healing time and be used as adjuvant therapy treatment to tide over drug resistance and has shown some promise in the healing of the corneal ulcers.

In this study 44 patients were studied. They were randomized into the Intervention group who were given the treatment of collagen cross linking along with standard medical therapy and the Control group who were given standard medical therapy along with a sham cross linking.

There were 20 patients in Intervention group and 24 patients in the Control group of which only one patient was lost to follow up in the Intervention group but 7 patients were lost to follow up in the Control group.

Therefore the final sample analyzed consisted of 17 patients in each group.

The average duration of time required for healing of the ulcers in both groups was assessed. It was found that there was no difference in the time taken to heal in both groups, with an average of about 30 days required for healing of ulcer in both groups. The addition of collagen cross linking to standard care as an adjuvant treatment did not help in reducing the time of healing of corneal ulcers.

Additionally, in this study 4 out of the 17 patients in both the arms of the study had a poor outcome. In the Intervention group, 3 patient developed perforation of the corneal ulcer and one patient developed thinning of the ulcer more than 50 %. In the control group, 3 patients developed corneal perforation and one patient had to undergo therapeutic keratoplasty.

Thus there was no difference in the failure rates in both of the arms of this study.

Two patients complained of increased pain following CXL. However, it cannot be commented whether CXL contributed to increased pain, as this study was not designed to assess the pain scale in patients undergoing cross linking.

In our pilot study the duration required for healing of ulcers in the group who had undergone cross linking was 21.6 days as compared to 48.7 days in the retrospective group who received only standard treatment. Even though statistical significance was not achieved ($p=0.06$) clinically a significant difference in the time of healing was noticed.

In the present study, one reason for not finding any benefit of CXL in reducing the Time to Healing may be the overwhelmingly large number of fungal ulcers compared to bacterial ulcers in the intervention arm (95%).

It has been suggested by several previous studies that CXL does not work as well for fungal ulcers, as opposed to bacterial ulcers(7)

The same study done on corneal ulcers of bacterial etiology may well show a better response to CXL

SUMMARY

Corneal ulcer is a sight threatening condition that can lead to corneal blindness following scarring. In some cases failure of treatment can result in corneal thinning, perforation, loss of eyeball and need for therapeutic keratoplasty. Most patients require microbiological investigation, in patient care and prolonged treatment for weeks. In some cases the ulcer is non healing and as well as antimicrobial resistant. Collagen cross linking is a procedure that strengthens the cornea and is a treatment used for keratoconus. Some antimicrobial property has been noted for the same and hence it is under trial in treatment of corneal ulcers.

Our pilot study conducted in 2014 showed clinically significant reduction in duration of healing post adjunctive treatment of corneal ulcers with CXL. The present study is a randomized control trial to study the effectiveness of crosslinking as an adjuvant therapy in healing of ulcers. 44 patients were recruited fitting the inclusion criteria and were randomized to an Intervention group and sham cross linking (Control) group. 17 patients in each group completed treatment. All patients received topical antimicrobial therapy. Within 48 hours of enrollment either crosslinking or placebo treatment was started. Each patient in the study group received maximum of three sessions of cross linking. Symptom relief and time of healing were noted.

The results were compared between the Intervention group and the Control group. Both groups showed similar healing time of 29.85 days of healing of ulcers, as well as similar success and failure rates.

There was no difference in the time of healing between the two groups. (p value=0.918).

Hence, this study does not suggest that collagen cross linking adds any benefit to the time taken to for fungal corneal ulcers to heal.

LIMITATIONS OF THE STUDY

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1. Patients lost to follow up were more than expected. Hence the sample size was not achieved.
2. Sample size is too small to develop a standard operating procedure.
3. Primary investigator was not blinded.
4. Long term effect of corneal collagen cross linking could not be studied in the short time frame.
5. Bacterial and fungal ulcers were not separately studied.
6. All species of micro- organism causing ulcer were studied together and not separately.

CONCLUSION

1.Collagen crosslinking has no benefit in reducing the time of healing of ulcers.

2.CXL does not reduce or increase the failure rate of corneal ulcers

3. There was no correlation between size of ulcer/diabetic status/type of organism and the outcome of corneal ulcer treatment with CXL compared to controls.

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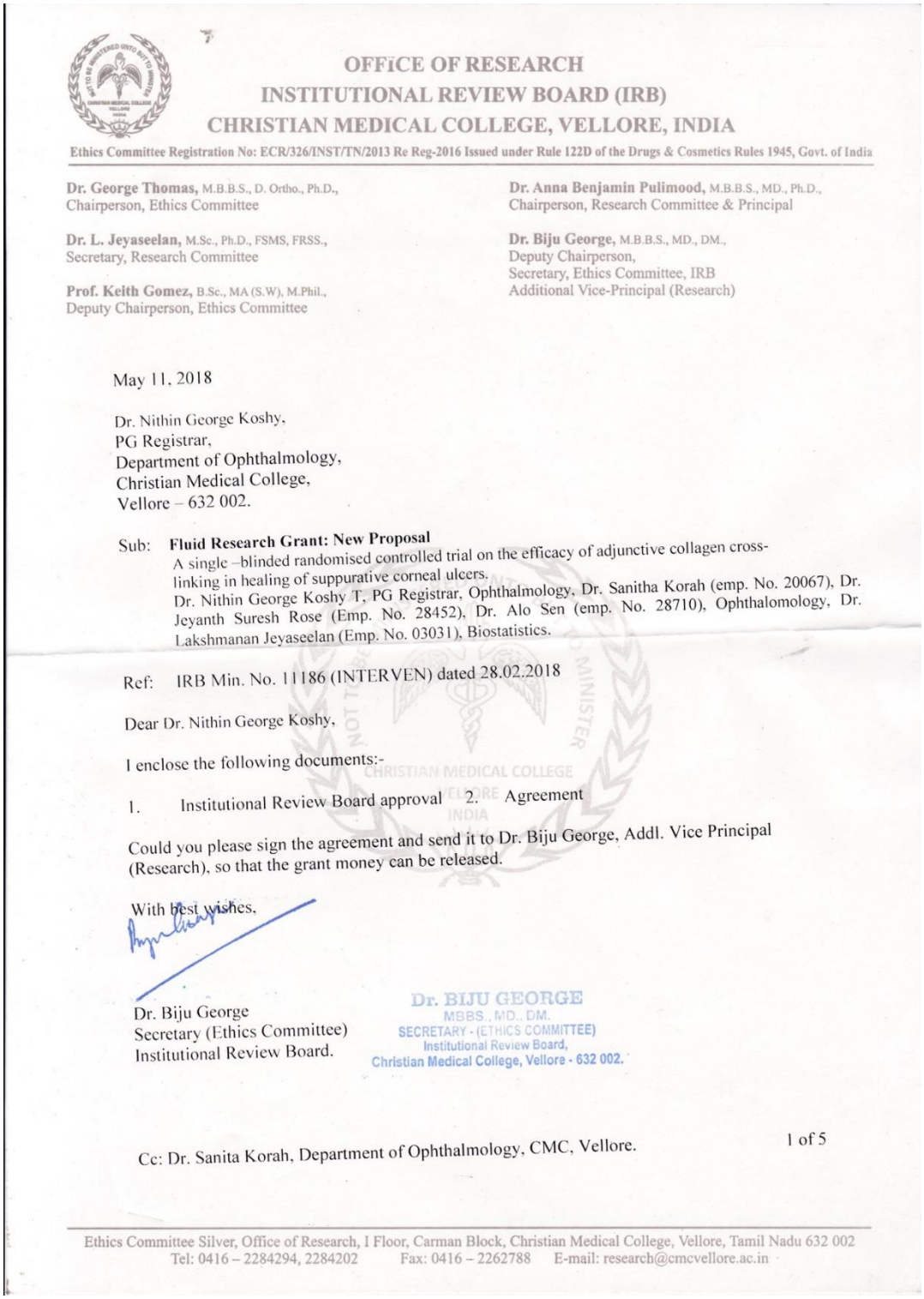
ANNEXURE

ANNEXURE 1

IRB APPROVAL FORM



OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA

Ethics Committee Registration No: ECR/326/INST/TN/2013 Re Reg-2016 Issued under Rule 122D of the Drugs & Cosmetics Rules 1945, Govt. of India

Dr. George Thomas, M.B.B.S., D. Ortho., Ph.D.,
Chairperson, Ethics Committee

Dr. L. Jeyaseelan, M.Sc., Ph.D., FSMS, FRSS.,
Secretary, Research Committee

Prof. Keith Gomez, B.Sc., MA (S.W), M.Phil.,
Deputy Chairperson, Ethics Committee

Dr. Anna Benjamin Pulimood, M.B.B.S., MD., Ph.D.,
Chairperson, Research Committee & Principal

Dr. Biju George, M.B.B.S., MD., DM.,
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

May 11, 2018

Dr. Nithin George Koshy,
PG Registrar,
Department of Ophthalmology,
Christian Medical College,
Vellore – 632 002.

Sub: **Fluid Research Grant: New Proposal**
A single –blinded randomised controlled trial on the efficacy of adjunctive collagen cross-linking in healing of suppurative corneal ulcers.
Dr. Nithin George Koshy T, PG Registrar, Ophthalmology, Dr. Sanitha Korah (emp. No. 20067), Dr. Jeyanth Suresh Rose (Emp. No. 28452), Dr. Alo Sen (emp. No. 28710), Ophthalomology, Dr. Lakshmanan Jeyaseelan (Emp. No. 03031), Biostatistics.

Ref: IRB Min. No. 11186 (INTERVEN) dated 28.02.2018

Dear Dr. Nithin George Koshy,

I enclose the following documents:-

1. Institutional Review Board approval 2. Agreement

Could you please sign the agreement and send it to Dr. Biju George, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes.

Dr. Biju George
Secretary (Ethics Committee)
Institutional Review Board.

Dr. BIJU GEORGE
MBBS., MD., DM.
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

Cc: Dr. Sanita Korah, Department of Ophthalmology, CMC, Vellore.

1 of 5

Ethics Committee Silver, Office of Research, I Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002
Tel: 0416 – 2284294, 2284202 Fax: 0416 – 2262788 E-mail: research@cmcvellore.ac.in

May 11, 2018

Dr. Nithin George Koshy,
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Ref: IRB Min. No. 11186 (INTERVEN) dated 28.02.2018

Dear Dr. Nithin George Koshy,

The Institutional Review Board **(Silver**, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project titled "A single –blinded randomised controlled trial on the efficacy of adjunctive collagen cross-linking in healing of suppurative corneal ulcers" on February 28, 2018.

The Committee reviewed the following documents:

1. IRB Application format
2. Information sheet Consent form and Patient Profile
3. Cvs. Of Drs. Annie Regi, Sravani, Anuja, Liji, Reeta, Swati, Ms. Rebecca.
4. Data Entry Form.
5. Good Clinical Practice Certificate
6. No. of documents 1- 5.

The following Institutional Review Board (Silver, Research & Ethics Committee) members were present at the meeting held on February 28th 2017 at 9.45 am in the New IRB Room, Christian Medical College, Bagayam, Vellore 632002.

Name	Qualification	Designation	Affiliation
Dr. George Thomas	MBBS, D Ortho, PhD	Orthopaedic Surgeon, St. Isabella Hospital, Chennai, Chairperson, Ethics Committee, IRB, Chennai	External, Clinician
Rev. Dr. T. Arul Dhas	MSc, BD, DPC, PhD(Edin)	Chaplaincy Department, CMC, Vellore	Internal, Social Scientist
Dr. Biju George	MBBS, MD, DM	Professor, Haematology, Additional Vice Principal (Research), Deputy Chairperson (Research Committee), Member Secretary (Ethics Committee), IRB, CMC, Vellore.	Internal, Clinician
Dr. L. Jeyaseelan	MSc, PhD, FSMS, FRSS	Professor & Head, Biostatistics, Secretary (Research Committee), IRB, CMC, Vellore	Internal, Statistician
Dr. Jayaprakash Muliyil	BSc, MBBS, MD, MPH, Dr PH (Epid), DMHC	Retired Professor, CMC, Vellore	External, Scientist &Epidemiologist
Prof. Keith Gomez	BSc, MA (S.W), M. Phil (Psychiatry Social Work)	Student counselor, Loyola College, Chennai, Deputy Chairperson, Ethics Committee, IRB	External, Lay Person & Social Scientist
Dr. P. Zachariah	MBBS, PhD	Retired Professor, Vellore	External, Clinician
Dr. Anuradha Bose	MBBS, DCH, MD,MRCP, FRCPCH	Professor of Paediatrics, Community Medicine, CMC, Vellore	Internal, Clinician
Dr. Sujith J Chandy	MBBS., MD., PhD., FRCP (E)	Professor, Clinical Pharmacology, CMC, Vellore	Internal, Pharmacologist
Dr. Ashish Goel	MBBS, MD, DM	Professor, Hepatology, CMC, Vellore	Internal, Clinician
Mr. Samuel Abraham	MA, PGDBA, PGDPM, M. Phil, BL.	Sr. Legal Officer, CMC, Vellore	Internal, Legal Expert
Mr. C. Sampath	BSc, BL	Advocate, Vellore	External, Legal Expert
Dr. Suresh Devasahayam	BE, MS, PhD	Professor of Bio-Engineering, CMC, Vellore	Internal, Basic Medical Scientist

Ethics Committee Silver, Office of Research, I Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002
Tel: 0416 – 2284294, 2284202 Fax: 0416 – 2262788 E-mail: research@cmcvellore.ac.in

Dr. Prasanna Samuel	MSc, PhD	Lecturer, Biostatistics, CMC, Vellore	Internal, Statistician
Dr. AbhayGahukamble	MS, D Ortho, DNB(Ortho)	Associate Professor, Paediatric Orthopaedics, CMC, Vellore	Internal, Clinician
Dr. Suceena Alexander	MBBS, MD, DM	Associate Professor, Nephrology, CMC, Vellore	Internal, Clinician
Dr. Sathya Subramani	MD, PhD	Professor, Physiology, CMC, Vellore	Internal, Clinician
Dr. Shirley David	MSc, PhD	Professor, Head of Fundamentals Nursing Department, College of Nursing, CMC, Vellore	Internal, Nurse
Mrs. Pattabiraman	BSc, DSSA	Social Worker, Vellore	External, Lay Person
Mrs. IlavarasiJesudoss	MSc (N)	Professor, Head of Medical Surgical Specialty 3 and Deputy Nursing Superintendent, College of Nursing, CMC, Vellore.	Internal, Nurse

We approve the project to be conducted as presented.

Kindly provide the total number of patients enrolled in your study and the total number of withdrawals for the study entitled: "A single –blinded randomised controlled trial on the efficacy of adjunctive collagen cross-linking in healing of suppurative corneal ulcers" on a monthly basis. Please send copies of this to the Research Office (research@cmcvellore.ac.in).

The Institutional Ethics Committee expects to be informed about the progress of the project, any **adverse events** occurring in the course of the project, any **amendments in the protocol and the patient information / informed consent**. On completion of the study you are expected to submit a copy of the **final report**. Respective forms can be downloaded from the following link: http://172.16.11.136/Research/IRB_Polices.html in the CMC Intranet and in the CMC website link address: http://www.cmch-vellore.edu/static/research/Index.html.

Ethics Committee Silver, Office of Research, I Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002
Tel: 0416 – 2284294, 2284202 Fax: 0416 – 2262788 E-mail: research@cmcvellore.ac.in

Fluid Grant Allocation:

A sum of 1,00,000/- INR (Rupees One Lakh Only) will be granted for 2 years. 50,000/- INR (Rupees Fifty Thousand only) will be granted for 12 months as an Ist Installment. The rest of the 50,000/- INR (Rupees Fifty thousand only) each will be released at the end of the first year as 2 nd Installment..

Yours sincerely,

Dr. Biju George
Secretary (Ethics Committee)
Institutional Review Board.

Dr. BIJU GEORGE
MBBS., MD., DM.
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

IRB Min. No. 11186 (INTERVEN) dated 28.02.2018

ANNEXURE II

INFORMATION SHEET

Christian Medical College, Vellore

Department of ophthalmology

Corneal Collagen Cross linking in corneal ulcers

Information sheet

The study that is being conducted requires the participation of patients suffering from corneal ulcers (Cornea – the anterior most transparent part of the eye). We are planning to conduct a randomized controlled trial on patients diagnosed with corneal ulcers. In this study the patients will be divided into two groups with the help of randomization so that the patient does not know to which group he belongs to. One group (interventional arm) will receive the present standard care along with a new treatment called collagen cross linking which is being studied. The other group (control arm) will receive the standard care along with a placebo treatment - using artificial light and tear substitutes.

The aim of the study is to determine whether collagen cross linking (a procedure that uses ultraviolet light in the presence of a dye) helps as an adjunctive therapy in reducing the healing time and outcome of corneal infections.

You are being kindly requested to participate in the above study.

The procedure for collagen cross linking involves exposure of the eye to

a specific amount of ultraviolet light onto the cornea for 30 minutes, in the presence of a photosensitising dye (riboflavin dye), which helps the UV light to

penetrate deep enough. The procedure has been found to be safe and free from side effects.

If required the procedure may be repeated, but not more than a maximum of three times.

All patients in the study will be receiving the standard therapy for the treatment of corneal ulcer. They will require hospital admission. Two days after admission if found suitable, they will be requested to enrol for the study and collagen cross linking will be started as adjunctive therapy. In order to assess

the treatment and for documentation clinical photographs of the eye will be taken.

Your participation in the study is entirely voluntary and you are also free to dropout of the study anytime you feel like. If you do so it will not affect your further treatment in the hospital in any way.

The information obtained from the study will help in analysing whether corneal collagen cross linking is useful in the treatment of corneal ulcer.

There will be no additional cost involved for you in the study and the procedure will be performed during the normal admission duration.

The results of the study will be published in a medical journal but your identity will not be revealed in any of the publication or presentation of the results.

However, your medical details may be reviewed by people involved in the study,

without your additional permission, should you decide to participate in the study.

If you have any further questions, please ask Dr Nithin George Koshy (Tel: 9497312596) or email: drnitkosh@gmail.com

ANNEXURE III

CONSENT FORM

Study Title: A SINGLE BLINDED RANDOMISED STUDY OF THE EFFICACY OF ADJUNCTIVE COLLAGEN CROSS LINKING IN HEALING OF SUPPURATIVE CORNEAL ULCERS

CONSENT FORM

Study Number: _____

Subject's Initials: _____ Subject's Name:

_____

Date of Birth / Age: _____

(i) I confirm that I have read and understood the information sheet dated

_____

for the above study and have had the opportunity to ask questions. [ ]

(ii) I understand that my participation in the study is voluntary and that I am free to

withdraw at any time, without giving any reason, without my medical care or legal

rights being affected. [ ]

(iii) I understand that the Sponsor of the clinical trial, others working on the

Sponsor's behalf (delete as appropriate), the Ethics Committee and the regulatory

authorities will not need my permission to look at my health records both in respect

of the current study and any further research that may be conducted in relation to it,

even if I withdraw from the trial. I agree to this access. However, I understand that

my identity will not be revealed in any information released to third parties or

published. [ ]

(iv) I agree not to restrict the use of any data or results that arise from this study

provided such a use is only for scientific purpose(s). [ ]

(v) I agree to take part in the above study. [ ]

Title of Research project:

Signature (or Thumb impression) of the Subject/Legally Acceptable

Date: _____/_____/_____

Signatory's Name: _____ Signature:

Or

Representative: _____

Date: _____/_____/_____

Signatory's Name: _____

Signature of the Investigator: _____

Date: _____/_____/_____

Study Investigator's Name: _____

Signature or thumb impression of the Witness:

_____

Date: _____/_____/_____

Name & Address of the Witness: _____

ANNEXURE IV

PATIENT PROFILE

PATIENT PROFILE

Name: Hospital number: Age:

Diagnosis: Eye:

Diabetes controlled/uncontrolled

Anaemia

Immunosuppression HIV/HBV/HCV

Smear report:

Fungal	Bacterial

Current Therapy

Antifungal	Antibacterial

Other Treatment

Glaucoma

POST ACCL

Session 1

Parameters	Grading		
	-1	0	+1
Reduction of pain			
Rounding of corneal infiltrates			
Reduction of hypopyon			
Reduction of size of epithelial defect			
A subjective 'forced gut feeling' of healing			
Total score			

POST ACCL

Session 2

Parameters	Grading		
	-1	0	+1
Reduction of pain			
Rounding of corneal infiltrates			
Reduction of hypopyon			

Reduction of size of epithelial defect			
A subjective ‘forced gut feeling’ of healing			

POST ACCL

Session 3

Parameters	Grading		
	-1	0	+1
Reduction of pain			
Rounding of corneal infiltrates			
Reduction of hypopyon			
Reduction of size of epithelial defect			
A subjective ‘forced gut feeling’ of healing			

POST ACCL

Session 4

Parameters	Grading		
	-1	0	+1

Reduction of pain			
Rounding of corneal infiltrates			
Reduction of hypopyon			
Reduction of size of epithelial defect			
A subjective ‘forced gut feeling’ of healing			

OUTCOME	YES	NO	TIME(in days)
1. Epithelial defect healing			
2. Corneal perforation			
3. therapeutic keratoplasty			
4. Corneal thinning>50%			
5. Pthysis			

ABSTRACT:

Title of the abstract : **A single-blinded randomised controlled trial on the efficacy of adjunctive collagen cross-linking in healing of suppurative corneal ulcers**

Department :Ophthalmology

Name of the candidate : Nithin George Koshy T

Degree and subject : M.S. Ophthalmology

Name of the guide :Dr Sanita Korah

OBJECTIVES:

Primary objective : To determine the benefit of adjunctive collagen cross linking (cxl) in reduction of the "time to healing" of suppurative corneal ulcer

Secondary objectives

A) to determine any difference in treatment failure rate (rates of perforation/keratoplasty/evisceration) of corneal ulcers treated with cxl as compared to the control group.

B) to assess the effect of risk factors (size of ulcer/diabetic status/type of organism)in outcome of corneal ulcer treatment with cxl compared to controls

METHODS: patients with suppurative corneal ulcers were admitted after routine microbiological analysis (scraping for smear and culture), and assessed on a daily basis to

determine response to treatment. All patients who fit the inclusion and exclusion criteria and were randomized tointerventional and control group.