USEFULNESS OF COLLAGEN CROSS LINKING (CXL) IN REDUCING THE TIME TO HEALING OF SUPPURATIVE CORNEAL ULCERS IN A SOUTH INDIAN TERTIARY CARE CENTRE

DISSERTATION SUBMITTED AS PART OF FULFILMENT FOR THE MS BRANCH III (OPHTHALMOLOGY) DEGREE EXAMINATION OF THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY, TO BE HELD IN APRIL 2015

BONAFIDE CERTIFICATE

This is to certify that this dissertation entitled "Usefulness of Corneal Collagen Crosslinking (CXL) in reducing the time to healing of suppurative corneal ulcers in a South Indian tertiary care center" done towards fulfilment of the requirements of the Tamil Nadu Dr. MGR Medical University, Chennai, for the MS Branch III (Ophthalmology) examination to be conducted in April 2015, is a bona fide work of Dr.Priya Basaiawmoit, postgraduate student in the Department of Ophthalmology, Christian Medical College, Vellore.

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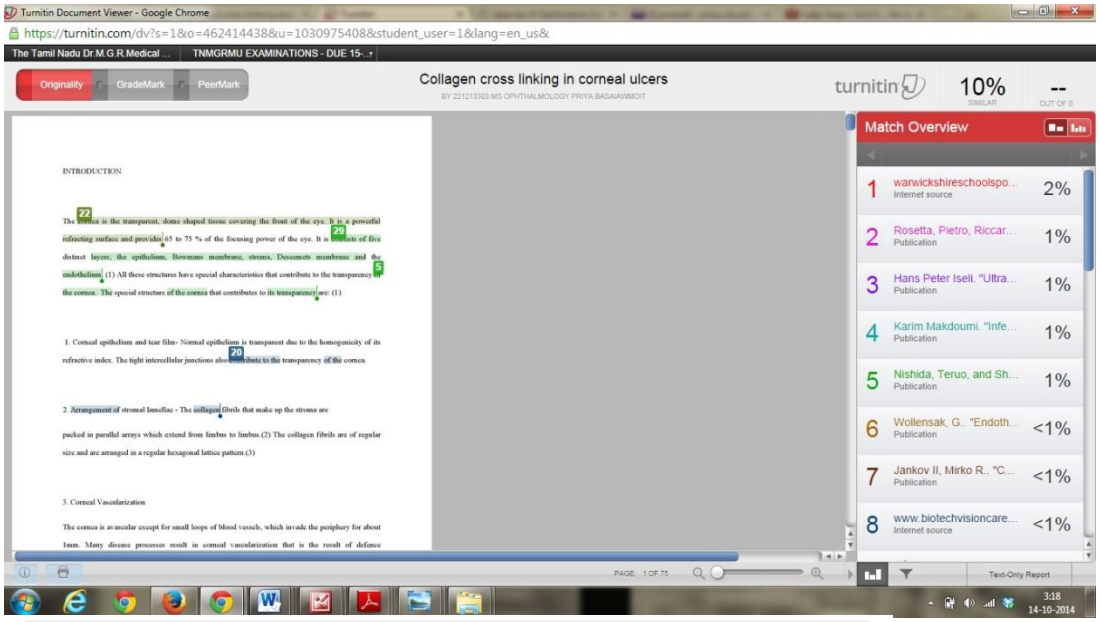
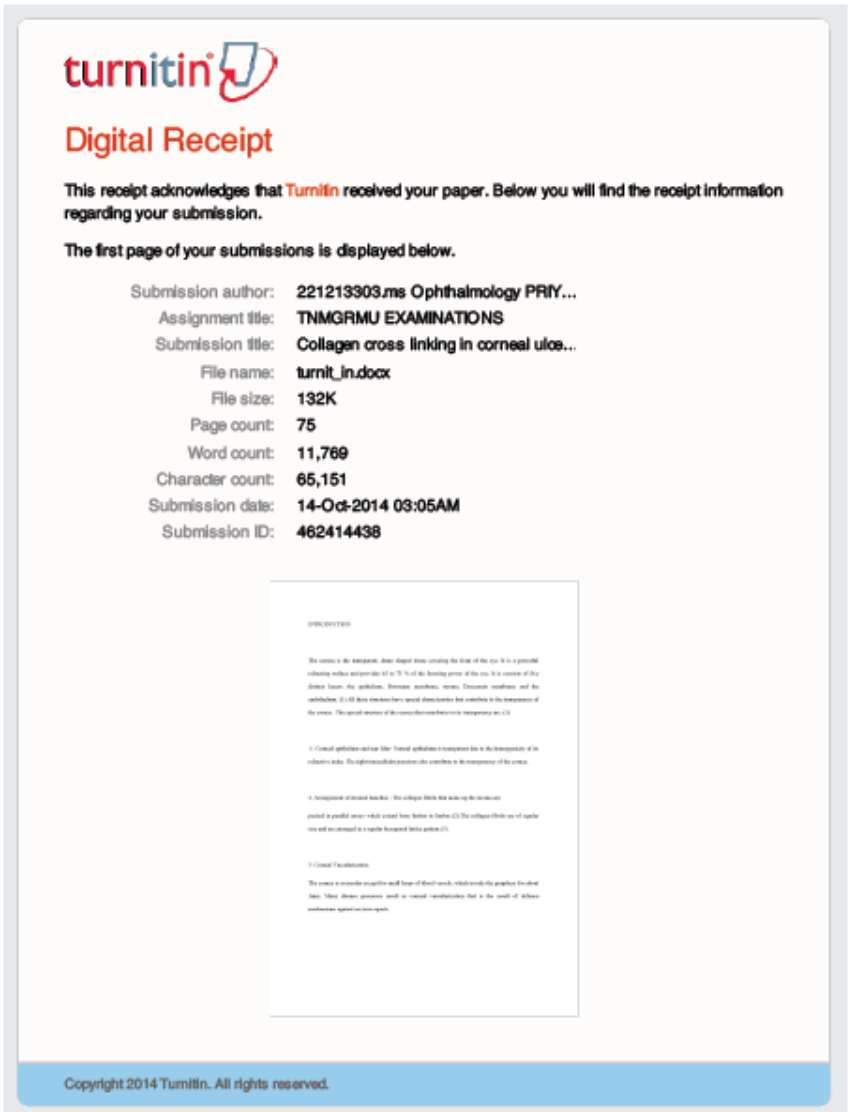
….and there are some " Angels without wings here on earth…" whom I can never thank enough…they know who they are…let it be like that!





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INTRODUCTION

INTRODUCTION

The cornea is the transparent, dome shaped tissue covering the front of the eye. It is a powerful refracting surface and provides 65 to 75 % of the focusing power of the eye. It is consists of five distinct layers; the epithelium, Bowmans membrane, stroma, Descemets membrane and the endothelium. (1) All these structures have special characteristics that contribute to the transparency of the cornea. The special structure of the cornea that contributes to its transparency are: (1)

1. Corneal epithelium and tear film- Normal epithelium is transparent due to the homogenicity of its refractive index. The tight intercellular junctions also contribute to the transparency of the cornea.

2. Arrangement of stromal lamellae - The collagen fibrils that make up the stroma are

packed in parallel arrays which extend from limbus to limbus.(2) The collagen fibrils are of regular size and are arranged in a regular hexagonal lattice pattern.(3)

3. Corneal Vascularization

The cornea is avascular except for small loops of blood vessels, which invade the periphery for about 1mm. Many disease processes result in corneal vascularization that is the result of defence mechanisms against noxious agents.

4. Corneal Hydration

The normal cornea maintains itself in a relative state of dehydration, which is essential for its transparency. The water content of the cornea is kept constant by a balance of factors which draw water in the cornea and those which draw water out of the cornea.

5. Cellular factors

Stromal keratocytes are a source of stromal collagens and proteoglycans, which are important for maintaining transparency. The cornea is highly innervated, but does not contain any blood vessels.(4)(5) Corneal injury can result in loss of this transparency and a patient may become blind just because of the corneal problem, while the rest of the eyeball is structurally and functionally intact. This condition is termed “Corneal Blindness”.

Corneal injury and corneal ulceration result in about 2 million new cases of corneal blindness annually.(6) Infectious keratitis, or corneal ulcer, is characterized by a corneal epithelial defect with underlying stromal inflammation and destruction caused by multiplying organisms and their toxins. Microbial keratitis is a leading cause of ocular morbidity and blindness worldwide.(6) Delayed or inappropriate treatment of infectious keratitis can lead to significant visual loss in as many as 50% of cases.(7) A large number of fungi, bacteria, protozoa, and viruses have been identified and implicated as infectious agents in microbial keratitis.

All microbial keratitis requires aggressive management to stop the disease process and reduce the extent of scarring which lead to loss of vision.(8)Although many of these cases resolve with empirical treatment using broad spectrum antibiotics (9)intensive empirical long term multidrug treatment has given rise to multidrug resistance of organisms. Such organisms alter the course of the disease and increase the morbidity. This has become a major public health concern. (7,8,10,11) Resistant strains are associated with relentless worsening of the corneal ulcer and marked visual loss. They lead to corneal degradation and melting resulting ultimately in perforation and loss of the integrity of the eyeball.

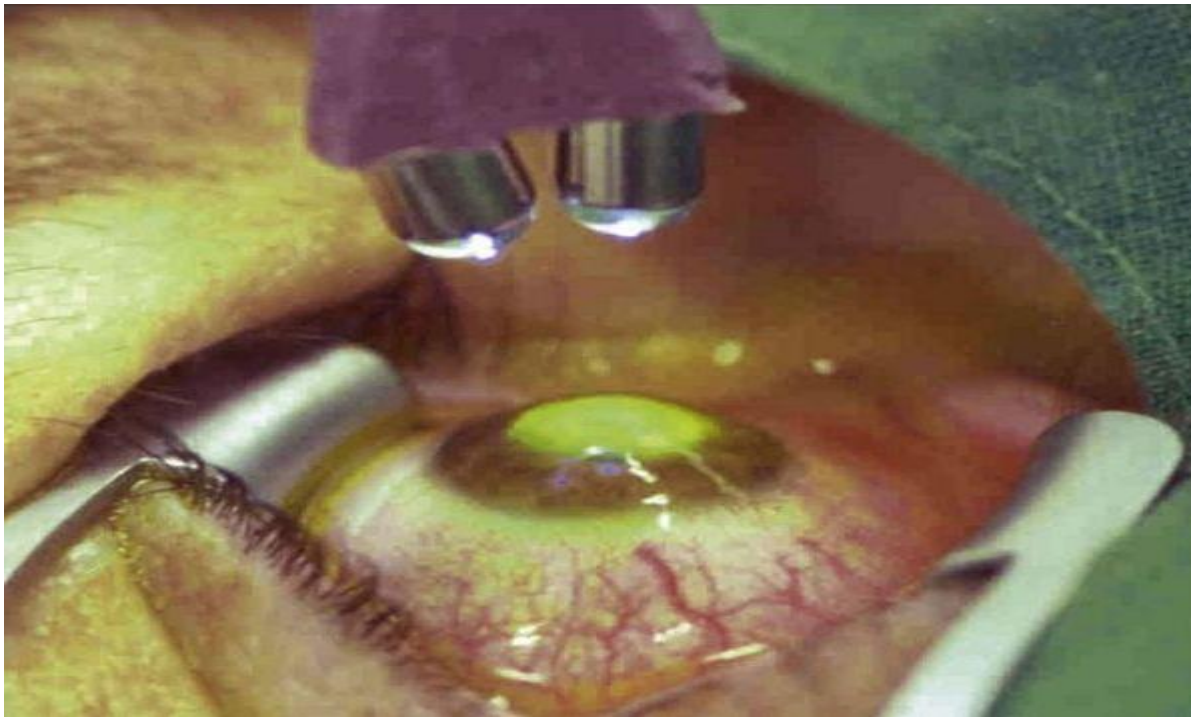
The frequency of drug resistance as well as the high cost of microbiological procedures to determine effective medication has led to a search for an alternative or adjunctive approach to therapy for resistant microbial keratitis. Corneal Collagen Cross- linking (COLLAGEN CROSS LINKING) may potentially be such a technique. Corneal Collagen Crosslinking was introduced in 1999 by Wollansak and Spoerl for the management of early keratoconus, a non-infective degeneration of the cornea which can progress to corneal blindness. COLLAGEN CROSS LINKING is now considered a standard procedure for prevention of progression of keratoconus, and is currently used worldwide.

Cross-linking in the cornea actually occurs as a natural biochemical process with aging in the normal cornea. It is responsible for the increased stiffness of the aged cornea as compared to a young (infant) cornea. “Collagen Cross-linking” is a procedure that results in faster and increased cross-linking which produces a stiffening effect, with no loss of corneal transparency, thus halting the progressive degeneration that occurs in keratoconus patients.

This therapy has proven safe for the cornea and is even used in children, with no long-term detrimental side-effects. (12,13) In the last decade, several in vitro studies and case-reports have been published which suggest a beneficial effect of CXL in the treatment of suppurative corneal ulcers. (14–16)

In vitro studies by Spoerl and others demonstrated that cross linked corneas have increased resistance against enzymatic digestion by pepsin and collagenases which enzymes produced by bacteria and fungi that precipitate corneal melting.(15–18) Additionally, Ultra Violet (UV) light and free oxygen radicals that result after cross-linking, may have an antimicrobial effect as they interfere with integrity of cell membrane.(19–21)

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.(22). This process using Riboflavin as a sensitizing agent in the presence of UV light has been found to be effective in inactivating pathogens in blood products and has been in use since 2000 for this purpose.(23,24)



Collagen Cross Linking

In contrast to Western countries, we in India have a much larger load of infective corneal ulcers (about 10 times more), and a much larger proportion of fungal ulcers,(25) for which availability of topical medication is limited compared to antibiotic drops for bacterial ulcers.

We postulate that CXL will reduce the "Time to Healing" as well as reduce the number of "Treatment Failures" (Loss of eyeball integrity), especially in the context of our country where adequate microbiological investigations may not be freely available or affordable to a large proportion of our patients. Most of the patients in our country who present to us with corneal ulcers as a result of their work as agricultural labourers, or "coolie-workers" are for a

low socio-economic stratum. A reduction in the time to healing will benefit our patients greatly as they will be able to get back to their work earlier if this treatment is successful.

CXL is a potentially valuable addition to our armamentarium for management of these cases.

AIMS & OBJECTIVES

Aim

Usefulness of Corneal Collagen Crosslinking (CXL) in reducing the time to healing of suppurative corneal ulcers in a South Indian tertiary care center.

Objectives

To determine if adjunctive corneal collagen cross linking (CXL) is useful in

1. Reducing the time to healing

2. Improving the outcome of suppurative corneal ulcers in a South Indian cohort of patients presenting with suppurative corneal ulcer, compared to a retrospective cohort of similar patients who have not received corneal collagen cross linking.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

INTRODUCTION

Corneal ulcers are a major cause of loss of vision in Asia with the largest number of reports originating from India.(6) Corneal ulcers are conventionally treated by topical, subconjunctival or intracameral antibiotics. However some cases are refractory to medical therapy, progressing inspite of maximal medical therapy. In order to preserve the eyeball, more invasive procedures such as conjunctival hooding or, more commonly, therapeutic penetrating keratoplasty need to be used. (6)

A relatively recent, non-invasive technique of corneal collagen cross-linking (CXL) has been developed over the last 15 years and been used successfully on corneal ulcers of different microbial origins. However, to my knowledge, there are only isolated case reports, or small case series in the literature, and data on the role of CXL in the treatment of corneal ulcers of fungal origin is even scantier.

This study aims to provide some additional information on the relevance and effectiveness of CXL in the management of suppurative corneal ulcers in the South Indian context, and hence addresses one of the major causes of blindness in India.

Here, I present the most recent and relevant bibliography that sets precedence for our study.

THE CORNEA

The cornea is the most important refracting element of the eye. It provides 65 to 75 % of the focusing power of the eye, It has a unique structure comprising of three distinct layers: epithelium, stroma and endothelium. Between the epithelium and the stroma lies a pseudo-basement membrane, the Bowman's membrane, which does not regenerate if injured. Healing in this structure occurs with scarring (1).

The endothelium lies on a basement membrane called the Descemet's membrane, which separates the stroma from the endothelium.

The corneal epithelium forms an effective mechanical barrier as a result of interdigitation of cell membranes and formation of junctional complexes such as tight junctions and desmosomes between adjacent cells.(1) Thus it protects against pathological agents and microorganisms.

The stroma comprises about 90% of thickness of the cornea.(1) It is made up of a very regular lattice arrangement of collagen fibrils which gives the cornea its strength, elasticity and form.(26) The unique molecular shape, Para crystalline arrangement and very regular fine diameter of the evenly spaced collagen fibrils is one of the major reasons for the transparency of the cornea. If damaged, this layer loses its regular lattice arrangement of collagen fibrils, and an opacity results. (27)

Keratocytes are the predominant cellular components of corneal stroma. They are spindle shaped cells which are scattered among the stromal lamellae which lie quiescent in the normal cornea. However, in response to an injury the keratocytes transform into myofibroblasts which produce smooth muscle actins, extra cellular matrix (ECM), collagen degrading enzyme, matrix metalloproteinase and cytokines for stromal tissue repair. These processes all result in a scar and loss of corneal clarity.

The cornea is densely innervated, but does not contain any blood vessels.(28)(29) This avascularity results in a the mechanism of wound healing in the cornea that is different from that elsewhere in the body. This avascularity is also a major factor contributing to the difficulty in antibiotic drugs reaching the corneal ulcers, resulting in prolonged time taken to heal.(30)

NORMAL PHYSIOLOGICAL CHANGES IN THE CORNEA WITH AGEING

Age-related crosslinking occurs in the stroma due to accumulation of Advanced Glycation Endproducts (AGEs). Corneal stroma differs from other collagenous structures in its transparency, which is the result of the precise organization of the stromal fibers and extracellular matrix. The collagen fibrils are arranged in parallel layers called lamellae. It has been demonstrated that enhanced AGE-mediated crosslinking could have benefits as a means for stiffening and strengthening the weakened cornea of patients with keratoconus.(31)

CORNEAL ULCER

A corneal ulcer is an epithelial defect with destruction, inflammation and infiltration of the underlying stroma. Corneal ulcers may be sterile or infective.

Microbial keratitis or infectious corneal ulcers are due to the proliferation of microorganisms. This could be bacterial, fungal, viral or parasitic.

Corneal ulcers are potentially sight threatening. Various soluble mediators produced by invading organisms and inflammatory cells against the invading organisms initiate focal inflammation. Macrophages invade the area to ingest the colonizing bacteria and degenerating neutrophils. Extensive stromal inflammation eventually leads to proteolytic stromal degradation and liquifactive tissue necrosis.The challenge for the clinician is not just diagnosis, but also appropriate therapy once diagnosis is established.

It is estimated that ocular trauma and corneal ulceration lead to 1.5 to 2 million new cases of corneal blindness annually in the world. (32) of which 90% occur in the developing nations.(33). The prevalence of corneal blindness in India is 0.45% which translates to 5.4 million people. (34) It has been observed that many of the corneal ulcers have some pathogenic microorganism associated with them. (35–40)

A study conducted in India on 5897 suspected cases of microbial keratitis found that most of the positive microbial keratitis cases were caused by bateria and fungi.(41) Fungal ulcers have been found to have poorer prognosis than bacterial ulcers .(41,42) Microbial keratitis of

bacterial and fungal origins are more common in the developing world than the developed world. (42) Fungal keratitis ranges from 4% -60% of infectious corneal ulcers in developing countries of the world other than India. (43–45) Comparatively, developed countries like the United States have a lower burden of fungal keratitis, even in the hot and humid regions of the country.(42)

When a patient presents with a corneal ulcer, there is not enough time to experiment with different medications. These ulcers require immediate attention and treatment especially if the ulcer is more than 2mm in size, if it is situated in the visual axis, if there is stromal melting, inflammation in anterior chamber or any scleral involvement. Any history of agricultural trauma particularly with vegetable matter is worrisome as it is a risk factor for fungal infection. History of contact lens wear or bathing in a common village pond may suggest acanthamoeba infection. The decision regarding appropriate treatment becomes more difficult when microscopy and laboratory tests are inconclusive or when the infection is mixed.

STANDARD TREATMENT OF CORNEAL ULCERS:

In many parts of the world, especially in the developing countries, microbiological investigations are not possible, or are not adequate. In such situations, broad spectrum topical medications are empirically used to control the corneal ulcer. For clinically suspected bacterial ulcers, topical Fluoroquinolones are widely used as empirical broad spectrum

antibiotics. In an attempt to ensure healing of the corneal ulcer, most independent practitioners tend to resort to the higher generation Fluoroquinolones,like topical gatifloxacin and moxifloxacin rather than risk treating the ulcer with lower generation antibiotics to which the organism may be resistant. (46)

Fungal keratitis is notorious for the difficulty in diagnosis and treatment. Advanced fungal infections may resemble advanced bacterial keratitis which can also lead to misdiagnosis . Fungi need to be cultured on special media and a long time is required for growth to occur in some cases. Additionally, antifungal sensitivity testing for fungal cultures is very restricted and not available in most centres. Added to this, antifungal drug formulations that are available for topical use on the eye are very limited.

Most of the currently available topical antifungal drugs have limited efficacy because of their poor penetration into the eye, limited spectrum of activity and surface toxicity. (47–49) Some deep fungal keratitis may become recalcitrant and non-responsive to topical and oral voriconazole therapy. Close patient monitoring is essential as spread of infection to the anterior chamber may worsen the prognosis.

Adjunctive approaches

Most adjunctive medical and surgical interventions for corneal ulcers focus on providing support with cycloplegia to reduce ciliary spasm, lubrication, collegenase inhibitors such as N

acetylcysteine, doxycycline as well as1000mg per day of oral Vitamin C. Uncontrolled corneal ulceration may lead to corneal perforation requiring cyanoacrylate glue, conjunctival flap or, as a last resort, therapeutic penetrating keratoplasty.

Ordinarily, keratoplasty is a sight-restoring procedure. However, in the setting of an aggressively progressive or perforated corneal ulcer, the corneal transplant has to be performed as an emergency procedure, on an intensely inflamed eye. This augers a poor prognosis for the graft survival and recovery of vision is rarely achieved. A second optical graft once the infection has healed has a much higher risk of graft rejection as the immune system is now primed to a foreign tissue.

Hence, the best case scenario for a patient with a corneal ulcer is to achieve healing of the ulcer with a scar that has no or minimal vascularization, with an intact eyeball. Such a scar is then eminently amenable to a planned optical penetrating or lamellar keratoplasty for restoration of vision. Hence from this perspective, the availability of any additional therapeutic options to treat a progressive corneal ulcer that is poorly responding to maximal conventional therapy is an added bonus for the patient, and will increase the chances of becoming eligible for a successful optical corneal transplant.

ROLE OF RIBOFLAVIN IN CROSS-LINKING AND ANTISEPSIS

Riboflavin (Vitamin B2) was discovered in 1927 by Paul Gyorgy and it was synthesized by Richard Khun in 1935. Initially, it was called Lactoflavin because of its high concentration in milk. Successively, a ribitol molecule was found and the name of vitamin B2 was changed to Riboflavin. It is a yellow substance barely soluble in water. It is heat resistant and highly fluorescent when excited by UV light.

Riboflavin has two important effects:

1. It absorbs UV radiation and acts as a photosensitizer for the generation of reactive oxygen species.

2. In combination with UV-A light Riboflavin forms radicals that cause cross linking. Dextran in riboflavin helps maintaining osmolarity and also avoids corneal soaking and swelling during treatment.

Riboflavin dye

UV-A light and riboflavin produce a photochemical reaction, there gives rise to covalent bonds or crosslinks in the corneal stroma. (50) In the 1960's it was discovered that riboflavin, when exposed to UV light, could inactivates the RNA of tobacco mosaic virus.(51) Later on, it was found that this combination can be used to inactivate a wide range of viruses, bacteria, and parasites.(23,52–56)

Photoactivation of riboflavin damages the RNA and DNA of microorganism by oxidative processes. This causes lesions in chromosomal strands. (51,57,58) When exposed to UV light the planer structure of riboflavin intercalates between bases of DNA and RNA resulting in oxidation of nuclei acids. It is also believed that there is a non-specific photochemical reaction causing oxidation damage to pathogen.(52,57) This antimicrobial effect of photochemical reaction is successfully utilized in the field of transfusion medicine, for inactivation of various microorganisms in blood products. (23,53)

MECHANISM OF EFFECT OF CROSS-LINKING ON CORNEAL MELTING

One of the most important mechanisms of corneal ulceration is the increased collagenolytic activity of tissue collagenases and collagenases of microbial origin which leads to corneal melting, perforation and loss of the eye. Bacteria and fungi can produce enzymes that can digest human connective tissue in the cornea inducing corneal melting.

It has been shown that collagen cross-linking helps in markedly increasing the resistance of collagen to these enzymes. Biomechanical studies done by Spoerl et al. showed that the rigidity of the cornea increased by about 72% in porcine corneas after cross-linking. In human corneas, it increased by a much larger amount of about 330% .(59) Riboflavin drops are used as a photosensitizer. This is followed by exposure to Ultraviolet-A. The collagen fibrils in the cornea form chemical covalent bonds in a process called photo polymerization.(60) This chemical bonding 'results in "cross-linking" and an increase in the strength, stability and rigidity of the corneal stroma, area of cross-linking and larger-diameter collagen fibres correlates with a band of high-molecular-weight collagen polymers

A second component of the assumed effect of cross-linking is the anti-microbial power of the cross-linking procedure itself. UV-A of wavelength 315nm to 380nm alone can inhibit the growth of bacteria and fungi. (61) Formation of the microbial cell wall is hindered by the free radicals produced during cross linking. (62)

LABORATORY STUDIES

Studies Demonstrating Increased Resistance of Cross-Linked Cornea to Melting

Many studies have established that a cross-linked cornea is resistant to collagen degrading enzymes produced by microbes.(63–65) One of the earliest studies on the effect of cross-linking on an ulcerated cornea was reported in 2004 by Spoerl et al.(64) This laboratory based study was done on enucleated porcine eyes and determined that photochemical crosslinking of the cornea using riboflavin and UVA substantially increased the resistance of the cornea to collagen digesting enzymes.(64) The authors found that the digestion time of pepsin, trypsin and bacterial collagenase doubled after crosslinking the corneas with riboflavin and UVA. They were thus able to prove that this treatment has both biochemical as well as biomechanical effects. The authors also observed, like previous studies on keratoconus patients by other researchers, that the cross-linking effect was localized to the anterior stroma, thus preventing photo-oxidative damage to the lens and corneal endothelium.

Studies Demonstrating Possible Cytotoxic Effects on Corneal Endothelium

Wollensak et al.(66) studied the possible cytotoxic effects of CXL on the corneal endothelium. This study was carried out on 34 rabbits and the endothelial cells were evaluated in histological sections after enucleation using the TUNEL technique. The study showed a specific threshold-like cytotoxic effect of CXL on the corneal endothelium starting

at an endothelial UVA dose of 0.65J/cm2. The authors then calculated that in human corneas of 400µM thickness this limit is reached using standard surface irradiance of 3.0mW/cm2. Wollensak et al. therefore suggested that CXL should not be carried out in patients with corneal ulcers and advanced keratoconus where there is corneal thinning beyond this corneal thickness limit. They suggest an alternative, cautious use of reduced dosage of 3.6 J/cm2 in corneas with at least 350µM thickness. The authors have strongly suggested the use of pachymetry before the CXL treatment to avoid damage to the corneas that are not thick enough to undergo the treatment safely.

In corneal ulcer patients however, this may not be as relevant as presumed because of the following facts.

1. UV light penetration into the cornea is limited by the ulcerated, moist and opacified corneal surface.

2. After the corneal ulcer heals, there is always a scar necessitating a corneal transplant to regain vision. Hence endothelial damage (which may occur even just because of the ulcer itself), is not as relevant in this situation where it is anyway going to be removed for the corneal transplant. This however does become relevant in a patient who will be undergoing an anterior lamellar graft.

In Vitro Studies on Microbiological Cultures

Martin et al. first reported in vitro studies of the effect of riboflavin and UVA on microbiological culture isolates in 2008. (14)

Two groups of organisms were studied:

Group 1: Pseudomonas aeruginosa, Staphylococcus aureus, and Staphylococcus epidermidis. Group 2: Methicillin-resistant S. aureus (MRSA), multidrug-resistant P. aeruginosa (MDRPA) and drug-resistant Streptococcus pneumonia (DRSP), and Candida albicans (CA). They found inhibition of growth of drug-sensitive as well as drug-resistant bacteria. They however did not observe any beneficial effect on the growth of Candida albicans.

Schrier et al(67) in 2009 have also reported the effectivity of a combination of riboflavin and UV-A light exposure of 30 min against Staphylococcus aureus and Pseudomonas aeruginosa.

In vitro studies by Makdoumi in 2009 tested the effect of riboflavin and UVA on Staphylococcus epidermidis, Staphylococcus aureus and Pseudomonas aeruginosa suspension in a fluid solution. They found that exposure for 60 minutes achieved a high degree of eradication of bacteria. Exposure of 30 min achieved only limited eradication. Kashiwabuchi et al. in 2011 studied the effect of UV-A and Riboflavin on fungal colonies of Fusarium solani and Candida albicans isolates. They however reported no beneficial effect of this treatment on these colonies. (68)

Jayesh Vazirani and Pravin K Vaddavalli carried out in-vitro experiments to test the effect of combination of riboflavin and UV-A on drug sensitive as well as drug resistant bacteria, fungi and Acanthamoeba. They found that the treatment was most effective against bacteria. Drug resistant strains of bacteria required multiple exposures. However, they did not find any arrest of growth of fungi or Acanthamoeba in vitro with this treatment.(69)

IN VIVO STUDIES

The following is a description of the available literature which suggests a beneficial effect of cross-linking in human eyes for corneal ulcers. As far as we know, there are only case reports or small case series (of upto 15 eyes) reported.

Karim Makdoumi et al. in 2010, reported successful treatment of seven eyes (six patients) with severe infectious keratitis using corneal cross linking. Duration of ulcer was from zero to seven days and all cases had corneal melting. They reported improvement of symptoms within 24-hours in 6 of the seven eyes. Corneal melting was arrested and complete epithelisation was achieved in all cases. Hypopyon present in two eyes regressed completely within 2 days after cross linking treatments. Patients were followed up between 1 month and 6 months. From their results, they concluded that CXL could be an effective treatment modality for recalcitrant infectious keratitis. (65)

They also suggested that this procedure may reduce the requirement of microbial analysis, and is especially useful in the scenario of high rates of antibiotic resistance which is currently prevalent.

The largest case series was reported by Rana Sorkhabi et al. in 2012. They did a clinical non-randomized case series of UVA riboflavin cross linking in resistant corneal ulcers. Ten patients with recalcitrant corneal ulcers were enrolled. Nine patients had staphylococcal ulcers, while the tenth had an aspergillus ulcer. None of the patients had shown any improvement with standard topical medical therapy. All the corneal ulcers were more than 3mm size, central with stromal infiltration and corneal melting. In all cases CXL was performed and patients were followed up as in-patients. Antibiotic drops were continued.

Eight patients (including the fungal ulcer patient) experienced improvement in symptoms of pain, epiphora and photophobia within 48 hours. The corneal melt reportedly stopped and corneal epithelialisation occurred without any additional treatment in these 8 patients over a period of 1week to approximately 3 months. The two other patients had deep stromal ulcers; one underwent therapeutic keratoplasty, and the other went on to evisceration. The authors therefore suggest that corneal ulceration in the deep stroma are probably not treated sufficiently with riboflavin photoactivation alone due to the limitation of light penetration and the micro-organisms embedded deeper than 300uM may well be protected from its effect.(70)

Hans Peter Iseli et al., from the Institute of refractive and ophthalmic surgery Zurich, Switzerland in 2008 reported 5 cases of infectious keratitis associated with corneal melting, which were resistant to topical medications and which were treated with CXL. The cultured organisms were Mycobacterium chelonae,2 cases of Non-tubercular mycobacterium, Acremonium and Fusarium.

CXL was performed when the patients did not respond to systemic and topical antibiotic therapy. Treatment consisted of one session of CXL using standard procedures. Following treatment, four out of five cases improved, with the infiltrate size and the melting process regressing. In the fifth case, a Fusarium corneal ulcer, progressive corneal melt resulted even after the Collagen cross linking, and an uneventful therapeutic keratoplasty was performed to save the eye. The authors, in retrospect, report that the continued melt was probably due to an immune response as there was no persistent fungal disease found in the excised corneal button. They suggest that this patient could actually have been treated with just steroids.

The authors recommend the CXL procedure in progressive corneal ulcers to avoid emergency keratoplasty. An additional advantage of elective corneal grafts is the lower graft rejection rates than that of emergency keratoplasty. Scheduled keratoplasty can also provide the opportunity to perform lamellar grafts. They also suggest that CXL may prevent microbial reinfection of the grafts. Additionally, they feel that antibiograms for slow growing bacteria such as Mycobacteria take time thus not allowing specific antimicrobial therapy to be applied on time. Thus, they report CXL effective in treating antibiotic resistant infections and melting keratitis.(71)

In a case report, Ferrari et al. (72) in 2009 describe a case of E.coli keratitis in a diabetic patient that they treated using CXL. One day after the procedure, the corneal ulcer was covered by cicatricial tissue with significant improvement of symptoms. Corneal oedema was almost completely resolved after a month with healing of the ulcer. With this study, the authors set precedence for treating cases with CXL where management of pain and infection is of extreme importance – here a case with pre-existing diabetes.

A case report by Moren et al. in 2010, described CXL as an effective treatment for severe infectious keratitis of unclear microbial origin. (73) Apart from analysis for various microbes, the authors carried out PCR analysis to rule out Herpes simplex type I and II viruses as UV-A activates these viruses. Within a few days of CXL treatment, the pain decreased and there was no more necrotizing process after the treatment. Two months after the procedure, the ulcer had completely healed. After 9 months, the cicatrical tissue faded, the cornea was more transparent and visual acuity improved to a level that there was no need for corneal transplantation or other surgical procedures.

Two case reports by Anwar et al. showcase the use of CXL in the treatment of infective keratitis refractive to antimicrobial therapy.(74) One of these patients had Staphylococcal keratitis while the other had keratitis caused by Aspergillus. In the first case, CXL was able to gradually reduce and heal the corneal abscess and epithelium. At 2 months there was a complete resolution of infection, but the vision was impaired by a central corneal scar. The second patient was considered for therapeutic penetrating keratoplasty due to the large size (9 x 7mm) of the abscess. To improve the outcome of the corneal grafting procedure by reducing the fungal load in the corneal abscess, CXL was performed 4 months prior to keratoplasty. The authors say that although the speed of resolution in these two cases was not as rapid as seen in the cases reported by Makdoumi et al, CXL was an effective procedure. They see CXL as a valuable asset for treatment of infectious keratitis that is refractive to antibicrobial therapy which also allows a more controlled and effective therapeutic penetrating keratoplasty procedure to be performed at a later date.

Yasin A.Khan et al. (75) from Wilmer Eye Institute in 2011 reported three cases of Acanthamoeba keratitis (two proven and one highly presumptive), which were unresponsive to medical treatment but were successfully treated with two sessions of adjunctive therapy using riboflavin and UVA light.

All the three patients were contact lens wearers in good health. All were referred to Wilmer Institute following unresponsiveness to medical treatment. Corneal ulcer scrapings mounted on 10% potassium hydroxide revealed Acanthamoeba cysts in two patients. Patients were prescribed 0.02% PHMB and 0.2% chlorhexidine gluconate applied hourly for the first 2 weeks. As there was no change in the symptoms and as the ulcer increased in size all the three patients received riboflavin with UVA exposure.

Symptoms of pain and photophobia reduced dramatically after 3 days of treatment. The ulceration gradually reduced after the first treatment with CXL and epithelial defects closed after 2 months of treatment. However, it was noted that the initial rapid rate of improvement started declining after 1-3 weeks. A second session of CXL was therefore applied when the ulcer sizes became static with no continued improvement documented. Following this, the ulcers again began to improve; the circumcorneal congestion cleared and symptoms resolved.

Complete closure of epithelial defects occurred 3-7 weeks after initial treatment. Corneal scars were left in 2 patients after complete resolution and penetrating keratoplasty was performed on them for restoration of vision. The third patient had a non-obstructive scar that was semitransparent, and hence keratoplasty was not required. There was no incidence of graft rejection in the patients who underwent keratoplasty, nor did infection recur in the third

patient. Histopathology of excised tissue did not reveal Acanthamoeba. Thus, the authors concluded that adjunctive use of UVA and Riboflavin therapy is a possible alternative for selected cases of medication resistant Acanthamoeba keratitis.

In contrast, Del Buey et al.(76) Conducted an in vitro study on Acanthamoeba isolates to evaluate the amoebicidal efficacy of CXL. This study is interesting and relevant in our context as amoebae have different morphological forms and so do fungi. Both have an environmentally sensitive form (trophozoite and mycellar forms respectively), and an environmentally resistant form (cyst and spore respectively). The authors show that 30 or 60 min of single UV-A exposure (3 mW/cm2, 370nm) or riboflavin therapy does not achieve eradication in 2 different acanthamoeba strains that they studied. Nevertheless, they mention that a single dose of 60 min may reduce growth of the organism and they suggest that longer exposures may be required to achieve desired effect. However, it is important to note that this is an in vitro study and may not entirely be representative of in vivo conditions.

Rohit shetty et al in 2014(8) reported collagen cross linking in 15 eyes of 15 patients with microbial keratitis who underwent collagen cross linking. Culture evaluation showed that nine patients had bacterial keratitis (Bacteria- staphylococci - 5 cases; streptococcus - 1 case; pseudomonas - 1 case;1 was gram negative inconclusive culture)and 6 had fungal keratitis, (Fungus- aspergillus – 3 cases , fusarium – 1 case, candida – 2 cases) All the patients were treated with antibiotics and anti fungals as appropriate. Those patients who did not respond to treatment underwent CXL as per standard protocol under topical anaesthesia. The same topical medication were continued after the cross linking. In this study, six with bacterial keratitis and three with fungal keratitis improved. Of the patients who did not show

improvement post-CXL, 3 were diagnosed with bacterial and 3 with fungal keratitis. Pseudomonas and streptococcus were the bacteria found in 2 of the patients with bacterial keratitis. The remaining one patient with bacterial keratitis had infection with a Gram negative organism with inconclusive culture report. Two of the three bacterial keratitis patients showed increase in hypopyon.

Of the 3 patients with no improvement post-CXL and fungal keratitis, 2 had candida infection with non-resolving ulcer and hypopyon and one had fusarium infection with a partial scar and non-resolving hypopyon. Pain and other symptoms improved in all patients. None of the patients have any post operative complications. He concluded that CXL is an effective procedure in non healing microbial keratitis

In a collaborative prospective study, Dalia G. Said(77) and colleagues from Cairo, Nottingham, UK and Geneva, Switzerland studied 40 patients with microbial keratitis. Twenty one patients underwent CXL and 19 were controls, who received only antimicrobial therapy. Slit lamp features, corneal healing time and complications were noted in each group and statistically analysed. The average healing time was 39 ± 18.22 days in CXL patients and 46 ± 27 days in the control group. Three patients in the control group had perforation and infection recurred in one eye. No complications were seen in the CXL group.

The group concluded that, though it did not shorten the healing time significantly, it is an effective adjuvant therapy in the management of severe microbial keratitis resulting in fewer complications. Recently, in 2001, Rosetta et al(78) introduced a new modification of the CXL

technique, the CXL-window absorption (CXL-WA) technique. They describe the safety and efficacy of treatment of infectious corneal ulcers with hypoosmolar riboflavin solution and CXL without de-epithalizing the cornea. Using this procedure, they treated 4 eyes of 3 patients with severe keratitis associated with corneal melting using this procedure. The infections (Pseudomonas, Acanthamoeba, Streptococcus pneumoniae respectively) in these patients were resistant to antimicrobial therapy and therefore this procedure was performed.

The modified procedure uses hypo-osmolar (instead of the standard isoosmolar) riboflavin 30 min before irradiation with UV-A without de-epithalialization. The penetration is obtained through the epithelial defect over the ulcer itself. This procedure can therefore be used for corneas that are thinner than 400µ. The hypo-osmolar solution induces stromal swelling and a consequent increase of thickness permits treatment. This also reduces the risk of delayed healing.

All the 3 patients reported in this article showed that the demarcation line was between 200 and 300µ, none showed adverse effects of swelling, and all had complete resolution of the infection. Furthermore, after 3 months, none of these patients showed any recurrence of infection at the 3-month follow up. The authors mention that stromal scars can persist in these cases with advanced infectious keratitis. They suggest that studies should be carried out to understand if better results in this aspect can be obtained by combining medical therapy with CXL when the infective process is first diagnosed. They also suggest further studies with

CXL-WA to find out the riboflavin level where peak eradication is achieved and if 30 min time is sufficient for complete sterilization of infectious ulcer with keratitis.

MATERIALS & METHODS

Materials and Methods

Study design

This was an Observational cohort study conducted at Department of Ophthalmology, Christian Medical College & Hospital, Schell campus, Vellore.

The study had two arms:

Retrospective arm: Historical cohort of patients who had suppurative corneal ulcers that satisfy the inclusion and exclusion criteria

Prospective arm: Cohort of patients with suppurative corneal ulcers who presented to our department and who satisfied our inclusion and exclusion criteria. These patients received adjunctive Corneal Collagen Cross linking therapy in addition to our current standard therapy.

Inclusion criteria

1. Adults 18 to 75 years of age

2. Corneal ulcer size 2-6mm

3. Smear or culture positive for bacteria or fungus

4. Patients willing for in-patient care

Exclusion criteria

1. Suspected viral keratitis
2. Suspected / proven Acanthamoeba keratitis
3. Corneal thinning of more than 50%
4. Known Pregnancy
5. Patients with history of previous collagen cross linking
6. Patients who are unable to understand and give consent
7. Charts that have incomplete data

Outcomes used for this study:

For both the arms of this study, the following were the end-point parameters used:

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

2. Non-healing (failure of treatment):

   A. Loss of the eyeball integrity (perforation, evisceration or shrinkage/atrophy of the eyeball)
   B. Emergency corneal transplantation (Therapeutic Keratoplasty)
   C. Withdrawal of patient from the study due to progressive thinning of the cornea >50%

Exposure in the Prospective Cohort of patients: Corneal Collagen Crosslinking

Sample size calculation

The time to healing was used to calculate sample size.

Our internal unpublished data has shown that the average healing time for a suppurative corneal ulcer is 7 weeks, with a Standard deviation of 7 days.

We postulated that the healing time with CXL would reduce by 7 days

Hence, if m1 is the mean healing time with no CXL, and m2 is the mean healing time with CXL,

$m1 - m2 = 7$ days

Using a $Z\alpha$ of 1.96 and a $Z\beta$ of 0.84, sample size was calculated using the formula:

$$n = \frac{2SD2\,(Z\alpha + Z\beta)\,2}{(m1 - m2)\,2}$$

$$= \frac{2 \times 7 \times 7\,(7.84)}{7 \times 7}$$

= 16 patients in each cohort

We decided to enrol 32 patients in the retrospective cohort to increase the power of the study.

Institutional review board

The study protocol was approved by the institutional review board and ethics committee of Christian Medical College, Vellore as per the ICMR guidelines required for any study conducted in this institution. A written informed consent in their own language was obtained from all patients who were recruited in the study.

Methodology

Retrospective arm:

For this part of the study, it was decided to use double the number of the calculated sample size in order to increase the power of the study. To this end, 32 consecutive charts of patients who had been admitted and treated in our department in the last three years, and who fit the inclusion and exclusion criteria were included for this study.

Data extraction was done and entered into an excel sheet for analysis.

Prospective Arm:

All patients who presented to our out-patients department with suppurative corneal ulcer during the study period were assessed for eligibility for this study.

Routine corneal scraping was done in all patients under aseptic conditions for microbiological examination of smears and for culture and sensitivity. Gram stain for bacteria and Lacto-phenol cotton blue (LPCB) for fungal hyphae was done. In addition, Fluorescent microscopy with Calcoflor – White stain was performed in cases where both the preceding smears were negative.

For culture, specimens were inoculated onto blood agar, chocolate agar, and Sabourad dextrose agar (SDA) media and were incubated in the Microbiology department for a total of 10 days to 2 weeks, depending on the media.

13 eyes of 16 patients with suppurative keratitis were successfully recruited for the prospective arm of this study.

After documentation of a detailed history and clinical examination, slit lamp photogragh was taken.

The parameters evaluated under slit lamp examination were:

- site and size of the infiltrate
- size of the epithelial defect,
- presence or absence of hypopyon,
- corneal thinning.

Standard medical therapy was started on all patients depending on the smear reports as follows:

Gram Stain positive:

Gram negative bacilli: Fortified Gentamycin drops (1.4%) + Cefazolin drops (5%)

Gram positive cocci : Fortified Gentamycin drops (1.4%) + Crystalline penicillin drops (100 000 units/ml)

All drops were started at hourly dosing.

LPCB/ Calcoflor – White positive for fungal hyphae:

Hourly Natamycin drops 5% +/- Tab. Ketoconazole 200mg twice daily (depending on the depth of the infiltrate)

Smear negative: Fortified Gentamycin drops (1.4%) + Cefazolin drops (5%)

If any other organism was suspected on the smear (Nocardia, Acanthamoeba, etc), appropriate treatment as per standard department policies were started.

All patient additionally received supportive therapy which included

Atropine sulphate 1% drops three times daily for relief of ciliary spasm

- Anti-infalmmatory painkillers and

- Anti- glaucoma medication if the intra-ocular pressure was high.

If the culture was positive, medication was altered if required as per the sensitivity profile.

All recuited patients underwent UV-A/riboflavin cross-linking (CXL) within 48 hours of admission. CXL was performed upto a maximum of four sessions, with an interval of 48 hours between therapy sessions. The time to healing (complete closure of the epithelial defect and regression of infitrate) or non-healing (loss of integrity of the eyeball, emergency corneal transplantation or progressive thinning of the cornea >50%) was determined. Thinning more than 50% was considered to increase the risk of endothelial damage.

TECHNIQUE FOR UV-A / RIBOFLAVIN COLLAGEN CROSS LINKING

The treatment procedure is performed under sterile conditions in an operating theatre. The currently accepted treatment protocol for treatment of keratoconus includes de - epithelialization for efficient penetration of riboflavin.(79) However, in suppurative keratitis, this step is not needed as there is already an epithelial defect over the corneal ulcer.

4% xylocaine topical anaesthetic drops are applied and loose epithelium and the debris are wiped away.

Riboflavin solution, 0.1% in 20% dextran, is then applied to the cornea every 3 minutes for 30

minutes. The saturation of the cornea with riboflavin is ensured by checking for the prescence of the greenish dye in the anterior chamber by slit lamp biomicroscopy blue light evaluation prior to UV-A light treatment.

The corneal ulcer is then irradiated for 30 minutes with UV-A light using an irradiance of 3 mW/cm2 with a surface irradiance of 5.4 J/cm2 . (Appasamy UVA light source). Throughout the duration of the procedure, the cornea is continually moistened with 0.1% riboflavin drops at 3 minute intervals and 4% xylocaine local anaesthetic drops as required.

After the procedure, the antimicrobial treatment that the patient was on is continued.

This treatment was repeated at 2 day intervals up to a maximum of 4 sittings.

All patients were assessed by an Ophthalmologist with at least 3 years of experience of treating corneal ulcers, on a daily basis using specific criteria to determine response to treatment.

In order to make this process as objective as possible, a grading system was developed to assess 6 aspects of corneal ulcer healing.

The indicators to assess improvement / non improvement were:

1. Reduction of pain

2. Rounding of corneal infiltrates

3. Reduction of height of hypopyon

4. Reduction of size of epithelial defect

5. Continued thinning of cornea

6. A subjective "forced gut-feeling"

A grading system of -1 to +1 (-1, 0, +1) was used for each of these indicators

-1: worsening

0: status quo

+1: improvement.

(A total possible grading of -6 to +6)

All these 6 parameters are routinely recorded in the in-patient notes of all patients admitted with corneal ulcers.

"The end points of "Healing" was determined by the assessing Ophthalmologist and was based on the closure of epithelial defect, scarring of the infiltrate and absence of corneal stromal inflammatory cells."

The number of cross-linking procedures done in each patient was decided on the basis of the healing rate. Those patients that were found to be healing well, were discharged earlier, and hence underwent less than four CXL procedures.

Data was entered in an excel sheet and analysis performed.

Appasamy UVA light source

Appasamy UVA light source

RESULTS

RESULTS

This study of corneal collagen cross linking(CXL) on suppurative corneal ulcers was conducted at the Department of Ophthalmology, Christian Medical College, Schell Campus, Vellore.

The study was conducted between April 2014 to October 2014. The treatment results following cross-linking in eligible patients was then compared to a retrospective cohort of patients who had presented with corneal ulcers to our department during the period between April 2013 to April 2014 and who did not undergo cross- linking.

The following are the results obtained.

NUMBER OF PATIENTS IN EACH ARM:

Retrospective Arm:

32 corneal ulcers were retrospectively enrolled in order to increase the power of the study.

Prospective Arm:

Although the sample size required for this study was 16 patients in each arm, only 13 patients who fit the inclusion and exclusion criteria for this study could be recruited to the prospective cohort during the study period. All 13 of these patients underwent collagen cross-linking while admitted in the ward.

Of these patients, follow-up till healing was complete only in 11 patients.

One patient became lost to follow up after discharge from the ward. He had received the maximum of four sittings of CXL.

One patient had not healed even at the completion of data collection for this thesis. He had also undergone the maximum of four sittings of CXL.

Of the remaining 11 patients, 10 patients healed and were classified as “Success of treatment”.

One patient perforated after 3 sittings of CXL, and was classified as “Failure of treatment”.

In order to analyse the “Time to Healing” in patients who had undergone CXL, as compared to patients who had not had this procedure, we took only the 11 patients for which complete follow-up was available.

For the rest of the data for the prospective arm, 13 recruited patients were taken.

DEMOGRAPHIC PROFILE OF ALL PATIENTS RECRUITED

Table 1: Gender profile of the Study Groups

	RETROSPECTIVE GROUP	PROSPECTIVE GROUP
Males	14	7
Females	18	6

Table 1 demonstrates the profile of patients in the study groups. There were more females in the retrospective group as compared to the prospective group.

The following pie charts are a pictorial representation of the gender distribution in the Retrospective cohort and the Prospective cohort.

Figure 1: Gender distribution – Retrospective cohort

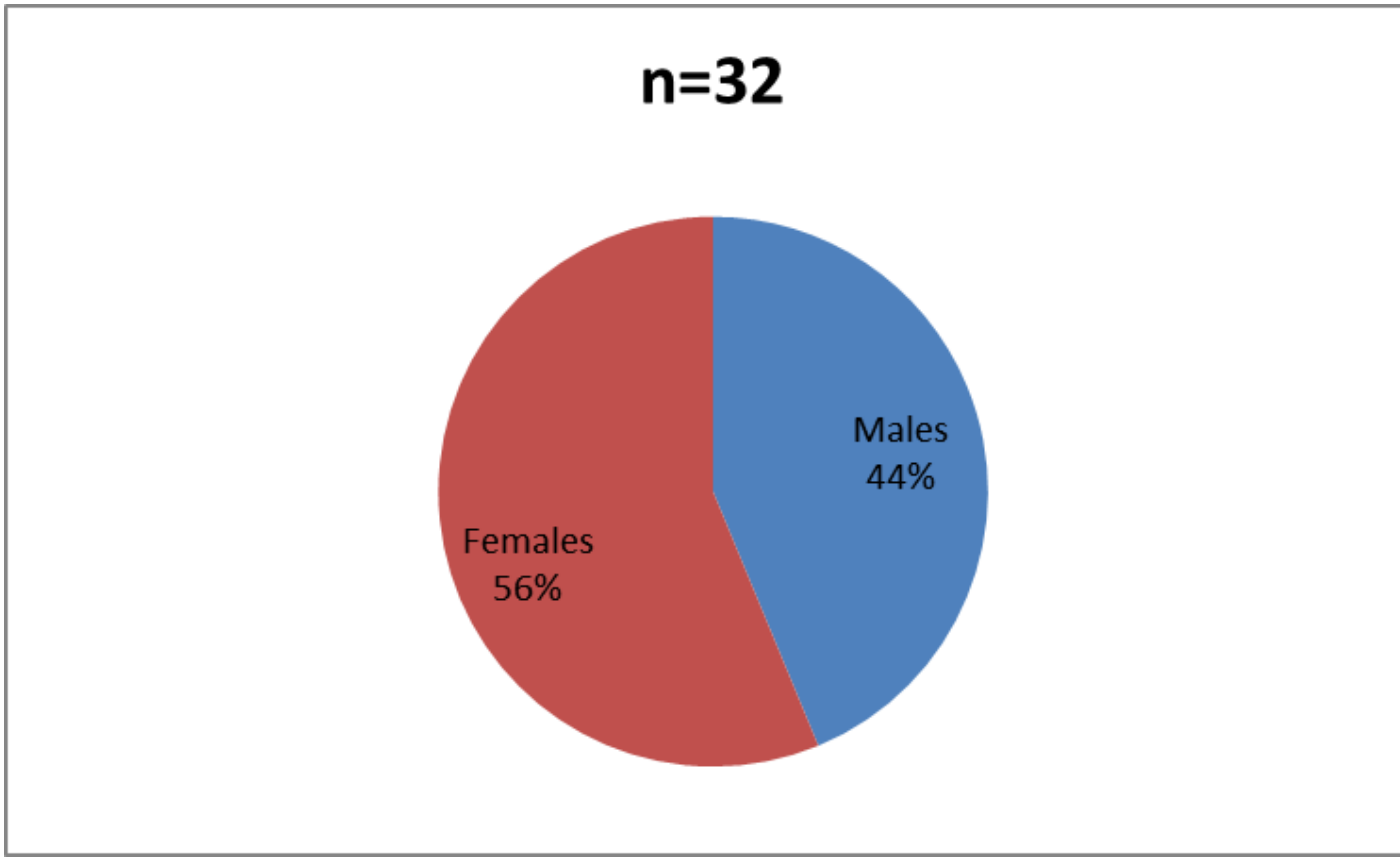


Figure 2: Gender distribution - Prospective arm

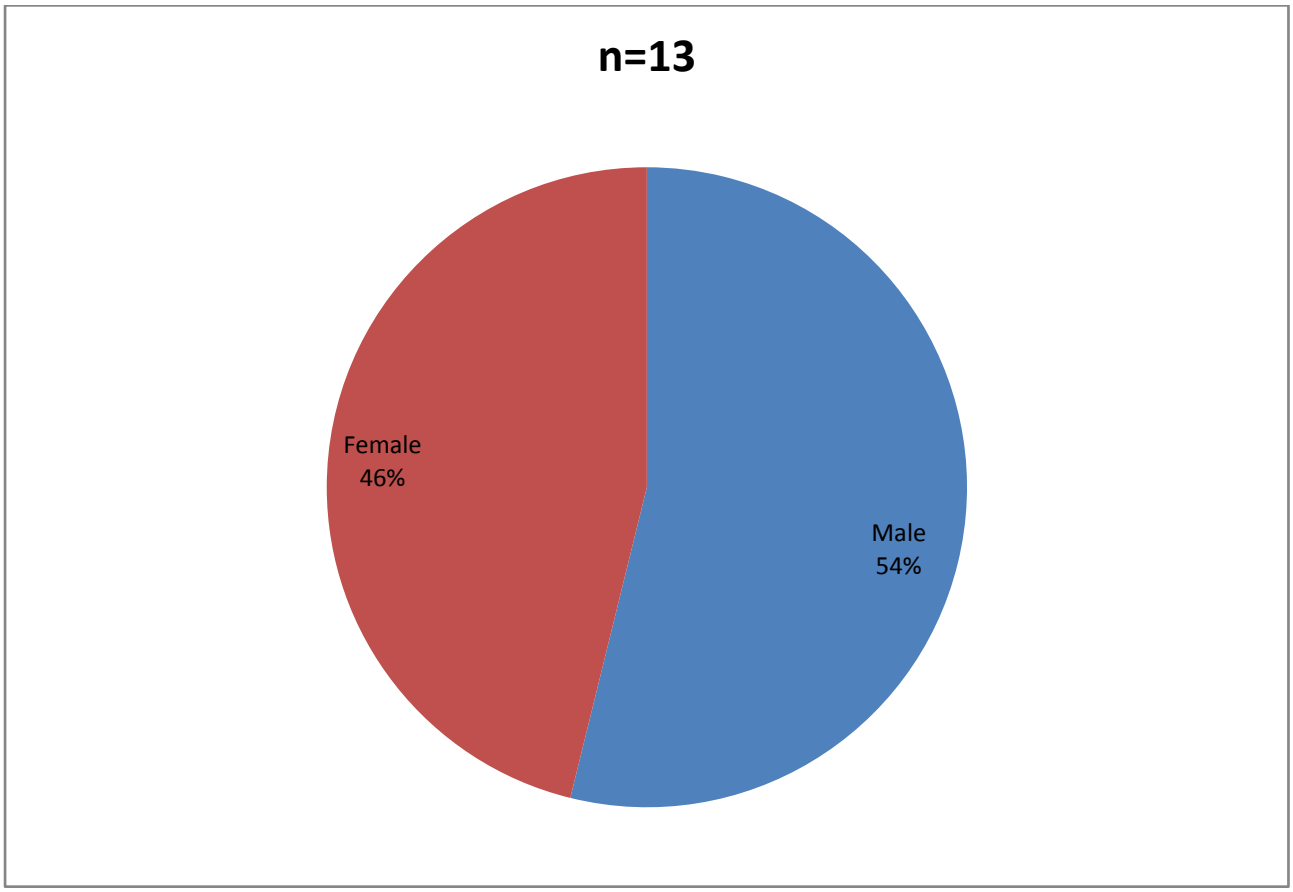
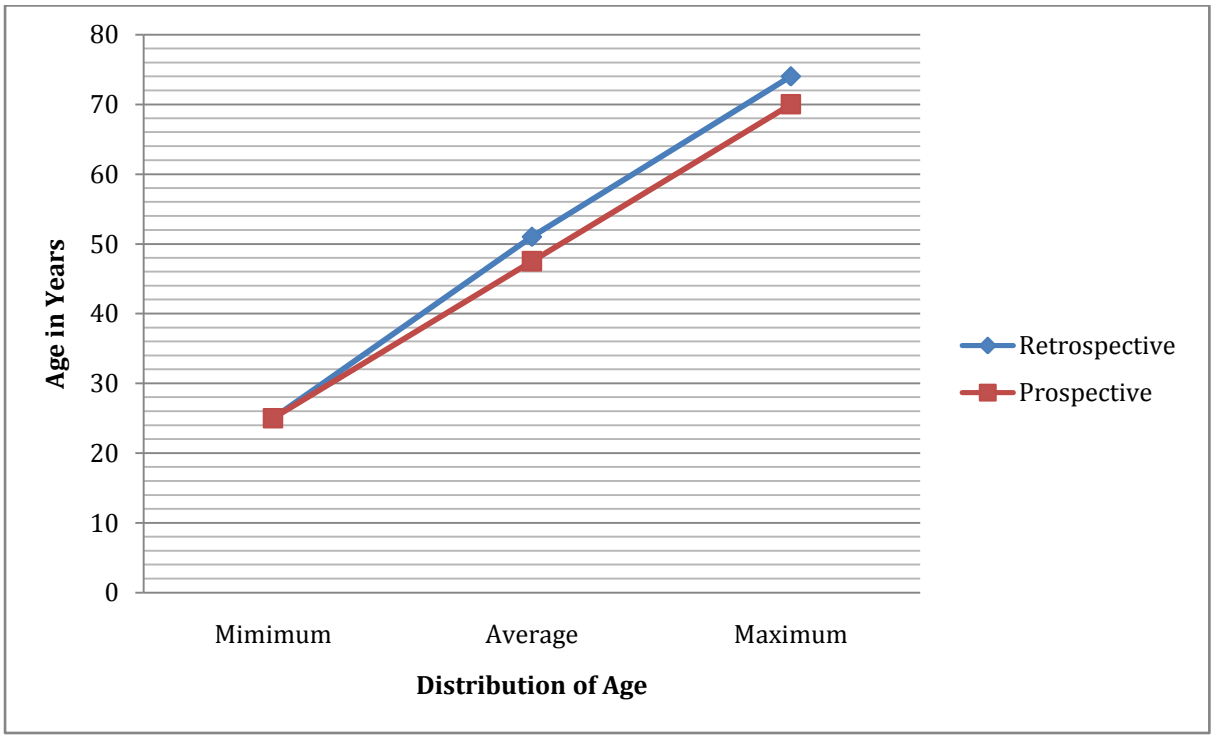


Table 2 gives the age distribution of the patients studied.

Table 2. Age Group Distribution of The Study Groups

	RETROSPECTIVE GROUP	PROSPECTIVE GROUP
Age	24 – 74 years	25-70 years
Mean age	51.0years	47.5 years

FIG 3: Graph Depicting Age Range with Mean Age in Both Groups



It can be seen that the age group in both the groups were very similar.

Tables 3 and 4 show the distribution of types of corneal ulcers in the two study groups, based on eitiology.

MICROBIOLOGICAL PROFILE OF ALL PATIENTS RECRUITED

Table 3: Type of Ulcer - Retrospective arm

Fungal ulcer	26
Bacterial ulcer	6

Figure 4: Retrospective arm – Type of Ulcer

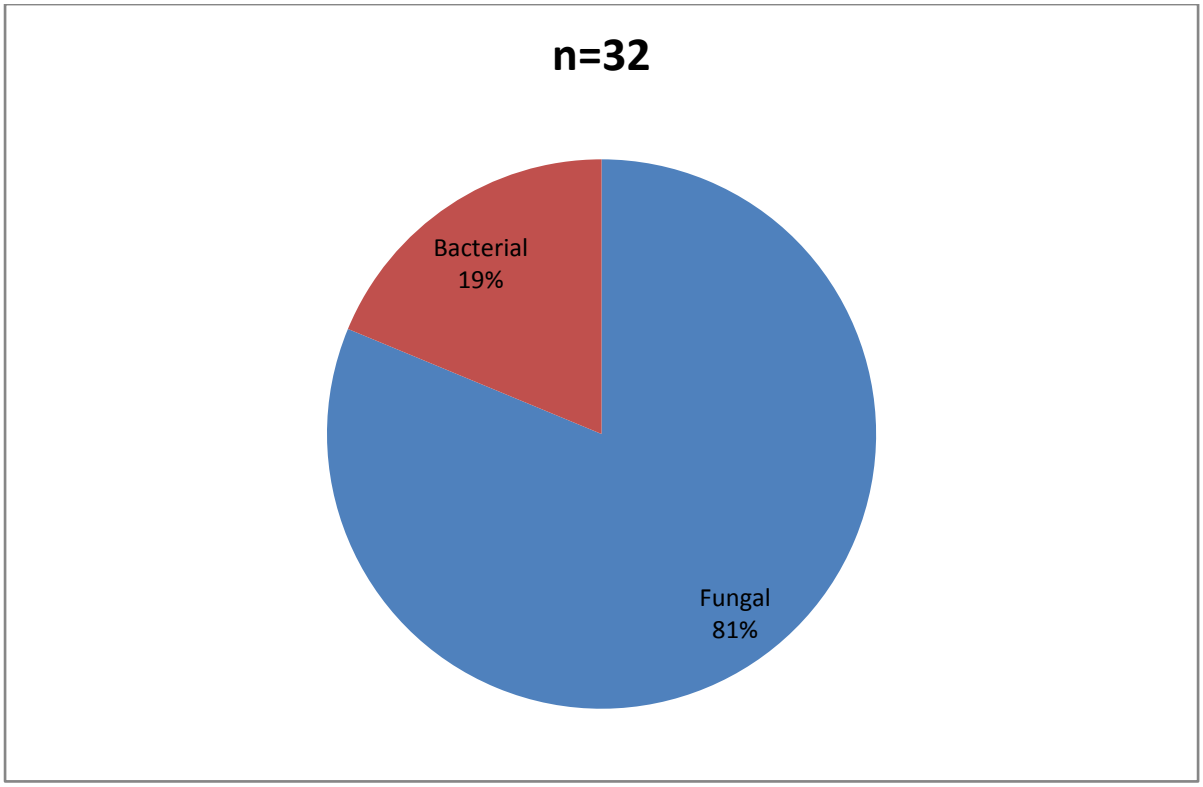
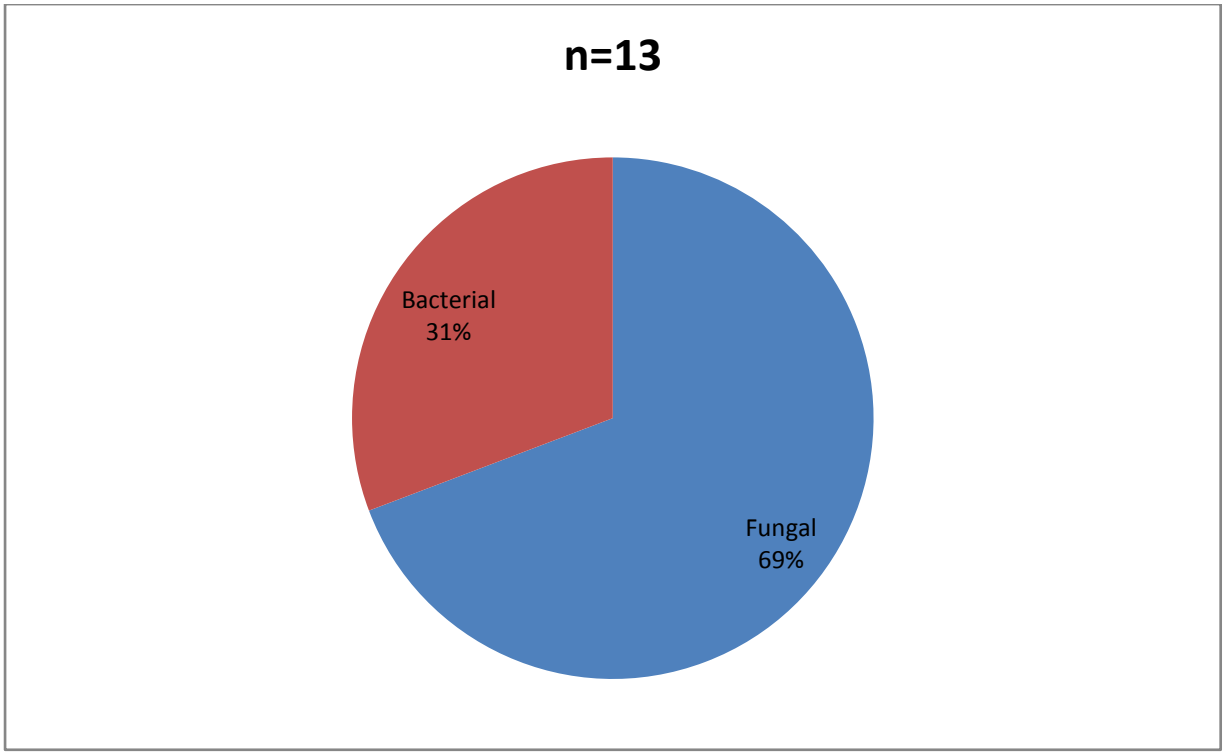


Table 4: Type of Ulcer - Prospective arm

Fungal	9
Bacterial	4

Figure 5: Prospective arm – Type of Ulcer

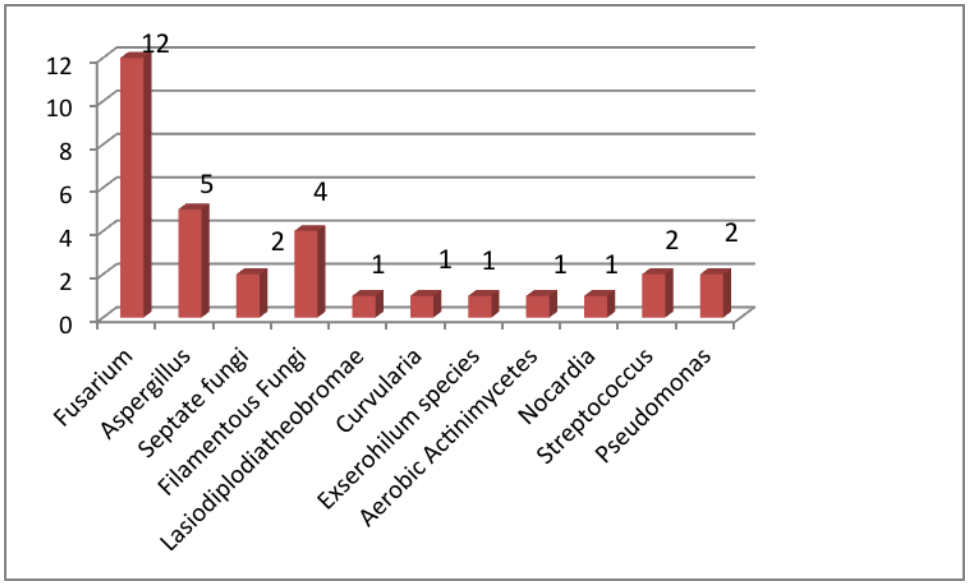


It can be seen that the retrospective arm shows 4 times more fungal, but prospective shows only about 2 times fungal as compared to bacterial.

This finding corresponds to the general South Indian data, where fungal ulcers outnumber bacterial ulcers, in contrast to the developed countries.(41)

Figure 6: Causative Organisms - Bacterial And Fungal Ulcers

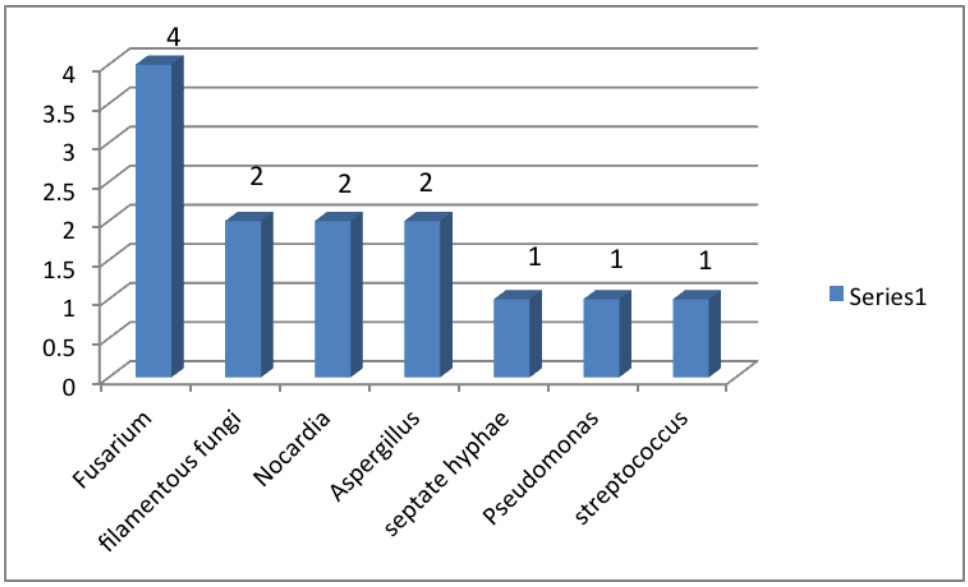
- Retrospective Arm



It can be seen that Fusarium ulcers were almost double in number as compared to Aspergillous ulcers, in agreement with other data from South India.

Figure 7: Causative Organisms - Bacterial And Fungal Ulcers

- Prospective Arm



In both the arms of this study, the most common fungal organism was Fusarium.

This is again in agreement with the South Indian data where Fusarium was found to be the commonest organism.(41)

Thus, most of the patients who underwent Collagen cross-linking were patients who had fungal corneal ulcers.

Size Of Ulcer:

The mean size of the ulcer in the Retrospective arm was 3.7 mm x 3.7 mm, (range: 1.4mm x 2.0mm to 6.0mm x 6.0mm) with hypopyon ranging from 0 to 3mm.

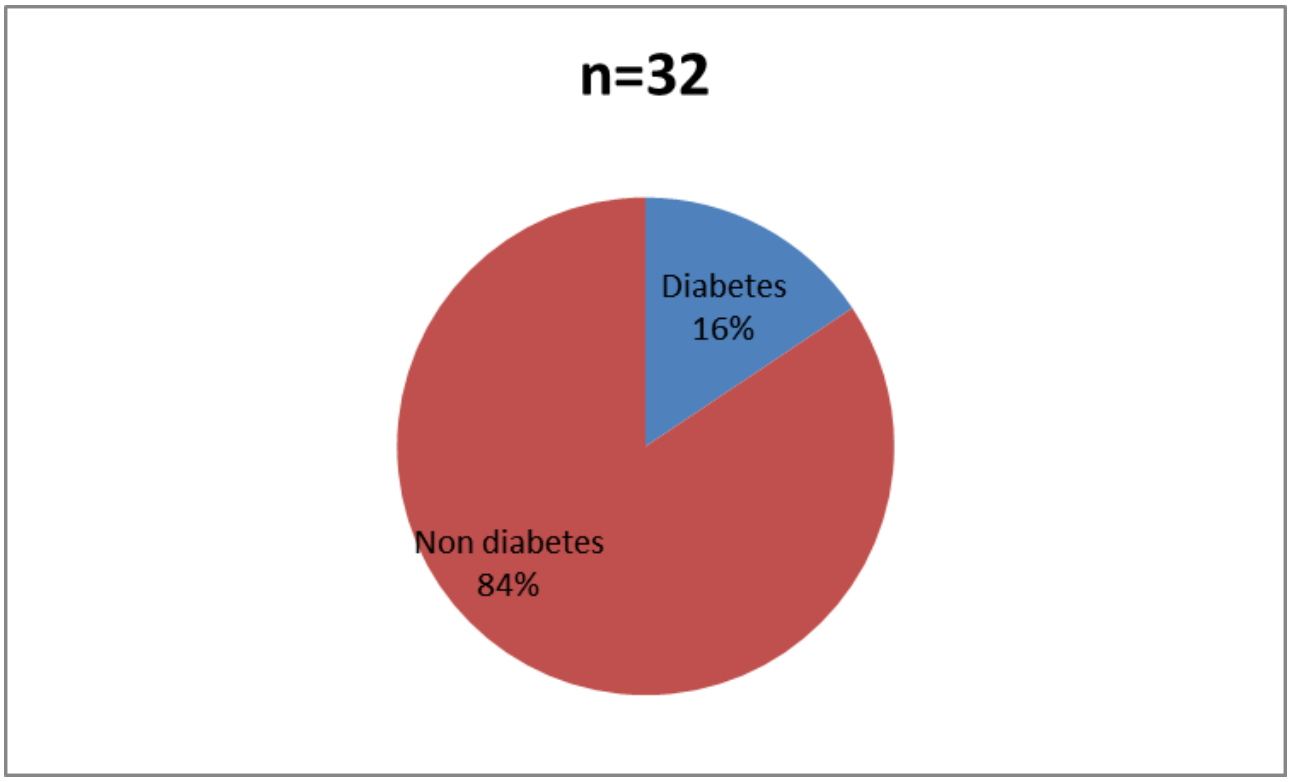
The mean size of the ulcer in the Prospective arm was 3.6mm x 3.53mm, (range: 1.1mm x 1.0mm to 6mm x 6mm)with hypopyon ranging from 0 to 3mm.

Table 5: Size and Hypopyon Range

	Retrospective Group	Prospective Group
Mean Ulcer Size (mm)	3.7 x 3.7 (Range 1.4 x 2.0 to 6 x 6)	3.6 x 3.53 (Range 1.1 x 1.0 to 6 x 6)
Hypopyon height (mm)	0 - 3	0 - 3

PREVALENCE OF DIABETES IN ALL RECRUITED PATIENTS

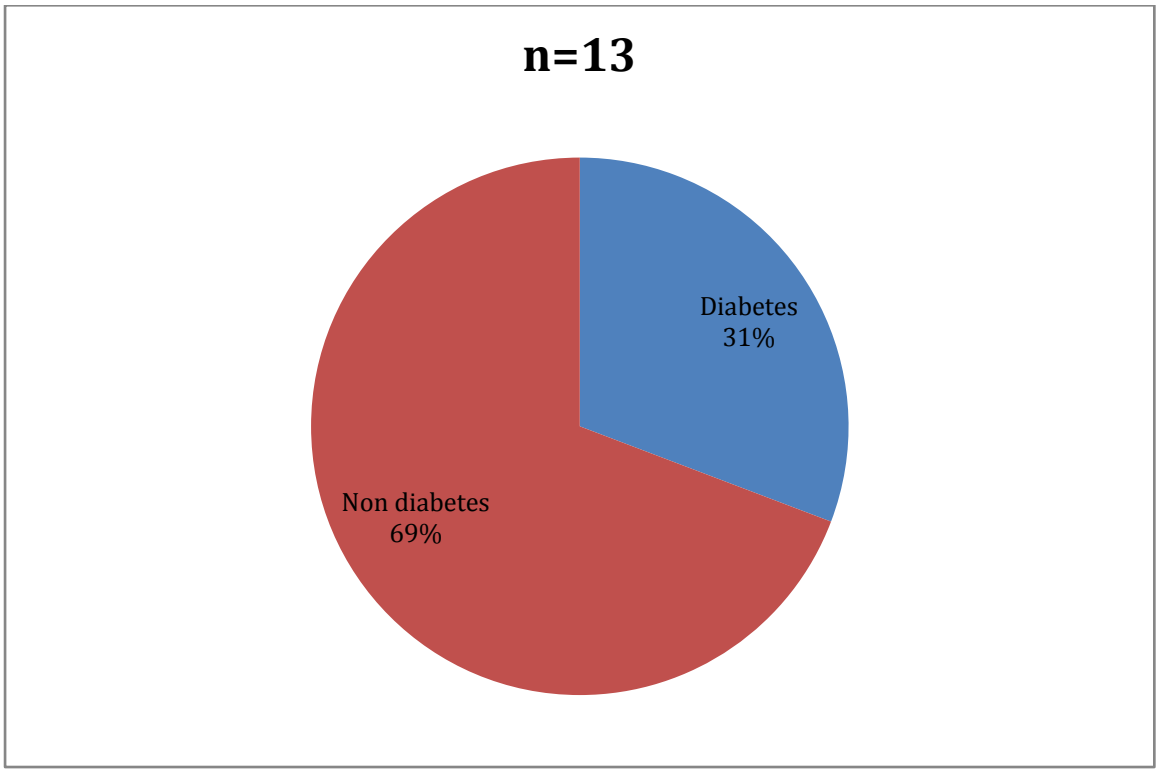
FIGURE 8: Incidence of Diabetes In Retrospective Arm



5 (15.6%) were diabetics, of which 3 were well controlled and 2 were uncontrolled when they presented to the department.

Of these patients, all but one healed without incident. One patient, who had presented with uncontrolled diabetes took a long time to heal (151 days)

FIGURE 9: Incidence Of Diabetes In Prospective Arm



4 patients had diabetes of which 3 were under control, and 1 had uncontrolled blood sugars. The uncontrolled patient was enrolled but had not healed at the conclusion of this study (35 days following recruitment)

The following table summarizes the data regarding the diabetics in each group.

Table 6: Number of Diabetics in Each Group

	RETROSPECTIVE GROUP	PROSPECTIVE GROUP
Controlled	3	3
Uncontrolled	2	1
Total	5 (out of 32 patients)	4 (out of 13 patients)

Table 7: Number of Diabetics in Retrospective arm with time to heal in days

RETROSPECTIVE ARM					
Parameter	Diabetes controlled			Diabetes uncontrolled	
Total Patients	3			2	
Healing time (days)	9	24	71	151	71

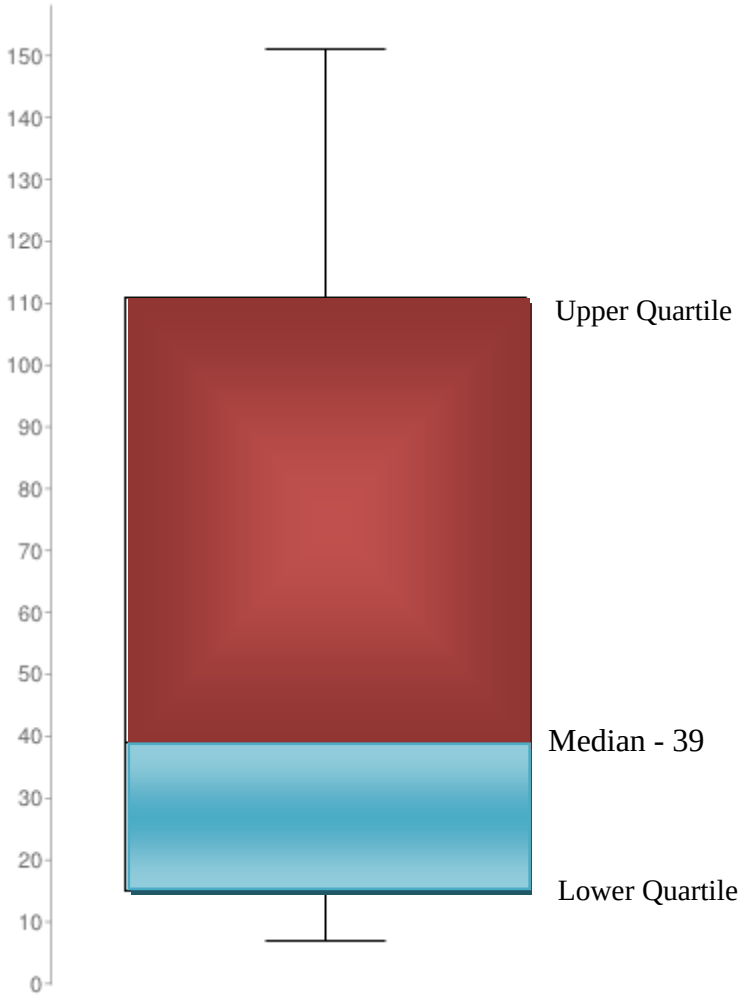
Table 8: Number of Diabetics in Prospective arm with time to heal in days

PROSPECTIVE ARM				
Parameter	Diabetes controlled			Diabetes uncontrolled
Total Patients	3			1
Healing time (days)	10	11	29	On follow up

TIME TO HEALING

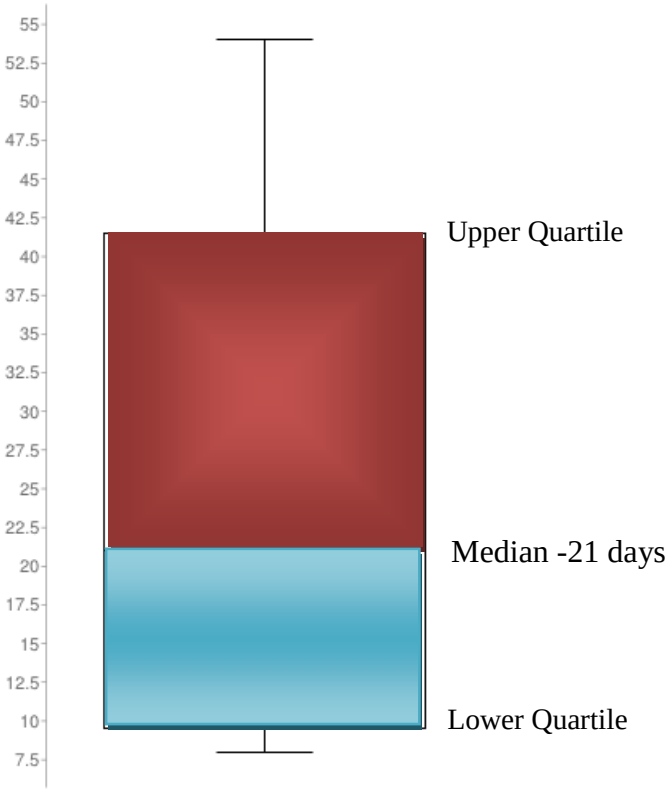
We analysed the data for time to heal among the retrospectiveandprospective arm using box and whisker plots which are represented in figure10 and 11

Figure 10: Box whisker plot of Retrospective group



The Median interquartile range time to heal was 39 days with a range of (8 -54) days. The range was between 7 days(minimum) to 151 days (maximum).

Figure 11: Box whisker plot of Prospective group



The Median interquartile range time to heal was 21 days with a range of (8 -54) days. The range was between 8 days(minimum) to 54 days (maximum).

Table 9: Number of patients with Successful Outcome, and Failure of Outcome

	Retrospective Arm	Prospective Arm
Success (Healed Ulcers)	28	10
Failures (Loss of eye)	4	1
Total	32	11

The patient in whom treatment failed in the prospective group perforated after three sittings of CXL. This patient had a deep stromal fungal ulcer with endothelial plaque.

As the time to heal was not normally distributed in each of the treatment type, we used the Mann- Whitney U test (Non parametric test) for Statistical Analysis.

Table 10:

	Retrospective Arm:	Prospective Arm:	Difference in mean between the groups
The Mean (Standard deviation) (in days)	48.8(36.7) days	23.2(14.3) days	25.6 days
The Median (Inter Quartile Range)	39(23-71)	21(11-29) days	

The difference in the healing time between the retrospective and prospective arm is almost statistically significant (P=0.06). Its trends towards the statistical significance

The healing time seen in prospective group that was treated with CXL was almost half that of the retrospective group.

Although the number of patients who had not received CXL was double that of those who had received CXL, the mean time to healing was still much lower in the CXL group.

This is a highly clinically significant result, in the context of this study where both groups had similar ulcer sizes, age groups , and organism profiles.

NUMBER NEEDED TO TREAT (NNT)

This intervention, in addition to reducing the "Time to Healing", also was found to reduce the risk of failure of treatment. We calculated the NNT for this reduction of risk of failure from Table 9 as follows:

Risk of failure in retrospective group: 4/32

Risk of failure in Prospective group (CXL group): 1/11

Absolute Risk Reduction = 4/32 -1/11

= 12/352

NNT = reciprocal of Absolute Risk Reduction = 352/12 = 29.33

Hence in order to prevent one failure, it is required to treat 30 patient with CXL. This does not seem to be an unreasonable number when failure of treatment means loss of the eye.

ADDITIONAL DATA

Table 11: Retrospective arm: Size of lesion,Hypopyon and healing time

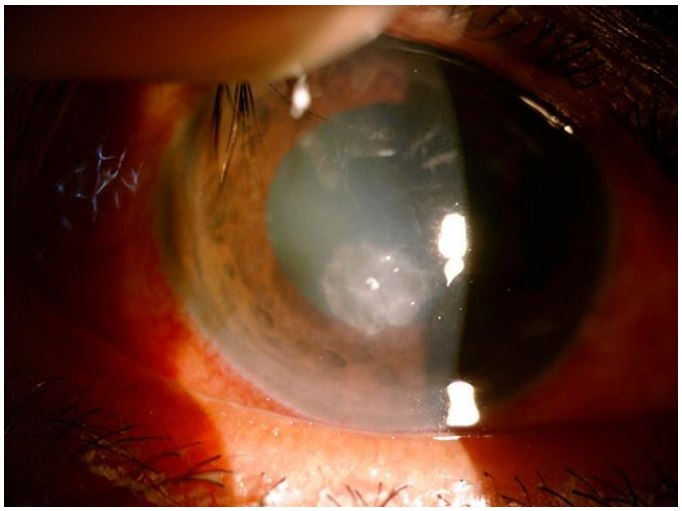
Serial No	Epithelial defect size	Hypopyon	Healing Time in days
1	4.5*4.5	No	7
2	2.6*2.6	1mm	22
3	1.4*2.0	No	9
4	5.3 *4.9	3mm	Nil
5	3.9*3.1	2.4mm	98
6	2.2*2.4	No	8
7	4.5*4.5	Yes,streak	8
8	3*2.9	1mm	28
9	2*3.4	1.3mm	36
10	2.7*2.7	1.2mm	25
11	4.5*4mm	Yes,streak	17
12	3.2*2.6	No	42
13	5.5*6.0	<1mm	Nil
14	4.7*4.1	1mm	24
15	3*3.2mm	1.2mm	78
16	3.2*3mm	<1mm	64
17	5.5*3.3	<1mm	44
18	3.5*3.5mm	2mm	151
19	5.1*4.9	No	122
20	3*2mm	1mm	16

21	5.4*4.9mm	Yes,streak	71
22	3.5*4.5mm	2.1mm	Nil
23	2.4*2.2mm	No	48
24	3.4*3mm	<1mm	25
25	2.8*1.6mm	No	78
26	3.6*2.4mm	<1mm	28
27	5.6*5.5mm	No	71
28	4.8*4.1mm	Yes,streak	93
29	5.5*4.8mm	No	29
30	6.0*6.0mm	<1mm	70
31	1.5*2.5	1mm	Nil
32	2.5*3m	1mm	54

Table 12: Prospective arm:Size of lesion,Hypopyon and healing time

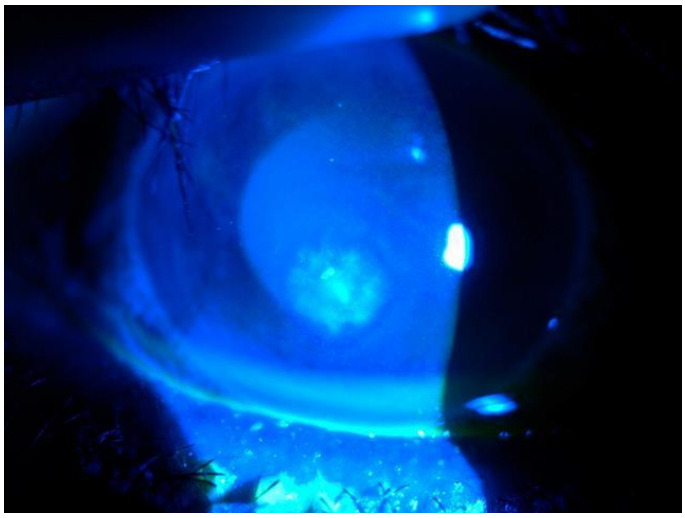
case	Epithelial defect size	Hypopyon	Healing Time in days
1	2*2.5mm	Nil	10
2	5*5mm	3mm	11
3	4.8*4.6mm	1mm	23
4	3.5*2.6mm	1.2mm	29
5	3.6*3.5mm	1mm	13
6	2.7*1.8mm	1mm	19
7	1.1*1mm	Nil	8
8	2.8*3mm	1mm	29
9	4.7*4.4mm	1mm	54
10	6*6mm	Nil	34
11	3.1 *3mm	1 mm	Perforated on 20^{th} day.

Serial Photographs of Patients who underwent Corneal Collagen Crosslinking (CXL)



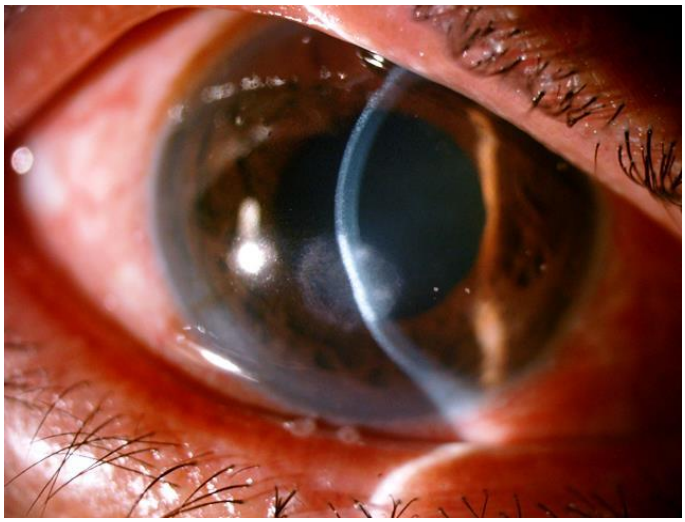
Case 1 A – Pre CXL

Causative organism- Nocardia



Case 1 B – Pre CXL

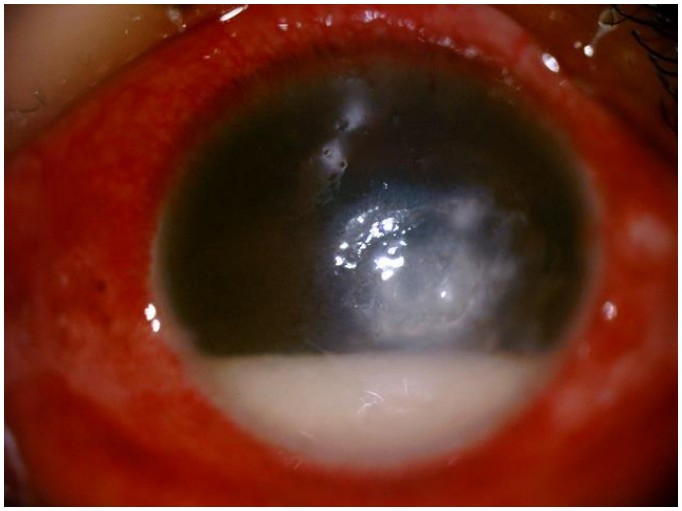
Causative organism- Nocardia



Case 1 C – Post CXL (Nocardia)

Time to heal- 7 days

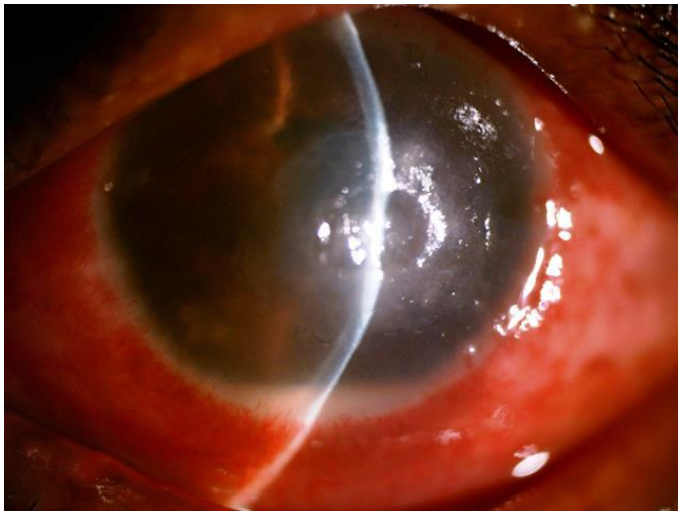
Scarring of the infiltrate



CASE 2 A PRE CXL

Causative Organism – Fusarium

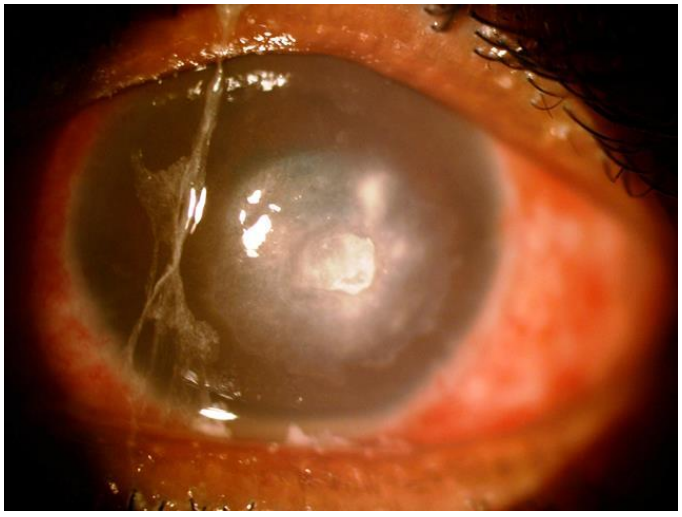
3mm hypopyon



CASE 2 B POST CXL

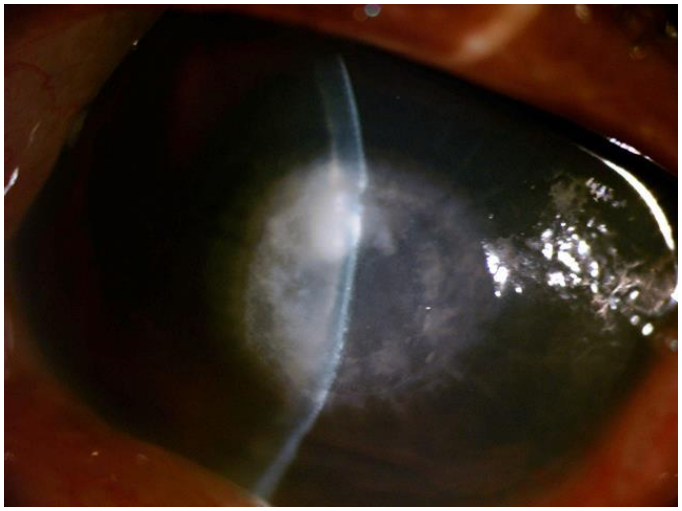
Resolution of hypopyon

Reduction in size of epithelial defect

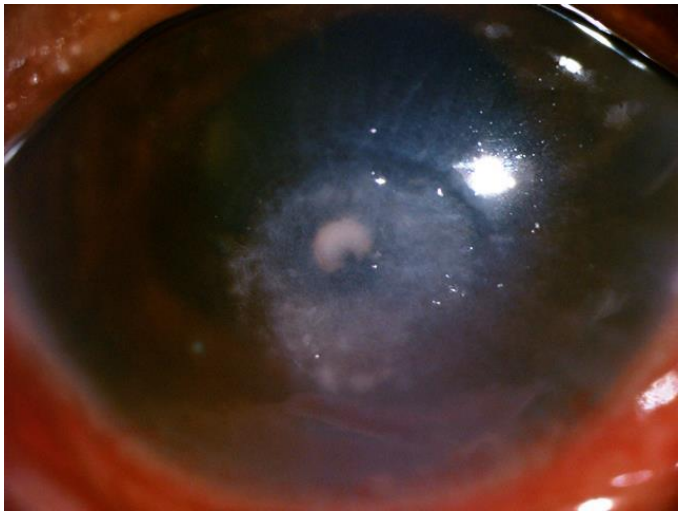


CASE 2C

Time to heal – 12 days



CASE 3 A



CASE 3 B

DISCUSSION

DISCUSSION:

Microbial keratitis or corneal ulcer, is defect in the corneal epithelium, associated with destruction and infiltration of the corneal stroma with inflammatory cells. In some case, as in autoimmune disorders, this ulcer is sterile and requires treatment with anti-inflammatory agents. However, in many cases, the ulcer is caused by pathogenic organisms.

The causative organisms can be bacteria, viruses, fungi or parasites. Patients present with acute or sub-acute onset of pain, conjunctival injection foreign body sensation. The ulceration can spread both circumferentially and through the full thickness of the cornea, leading to perforation.

A patient with a corneal ulcer ideally undergoes a battery of microbiological investigations inluding Smears for bacteria (Grams-stain) and Fungus (Potassium hydroxide- KOH, or Lactophenol Cotton Blue –LPCB), and culture plating in Blood agar, Saboraud Dextrose Agar and Chocolate Agar. If Acanthamoeba is suspected, Non-nutrient agar with E coli overlay is also used. Based on the results of the smear, and later the culture and sensitivity profile, appropriate topical medication is started and modified as required. Systemic anti-microbial medication is not used except in fungal ulcers where there is suspicion of hyphae infiltrating through the corneal endothelium into the anterior chamber.

Even with all these measures, the lack of vascularity of the cornea, the tight junctions of the epithelial and endothelial cells, as well as the lipid solubility/aqueus solubility issues of the medications used, results in difficulty in healing of the ulcer, usually necessitation weeks of in-patient care to get the ulcer under control. All corneal ulcers heal with a scar, as there is damage to the Bowmans membrane and underlying stroma.

The best case scenario for a patient with a corneal ulcer, is for healing to occur with no loss of the integrity of the eyeball, and minimal vascularization of the resulting scar. This results in corneal

blindness if the ulcer is in the pupillary axis, which can then be treated with a successful optical corneal transplantation.

There are several issues that limit this result, either leading to an even longer time for the ulcer to heal, or resulting in failure of treatment and loss of the eye.

One of the major issues is the very high cost of microbiological investigations, which makes it unaffordable to large sections of our population. Without proper microbiological sensitivity studies, acurate and directed antimicrobial treatment is impossible. In this situation, multiple broad-spectrum antibiotics, usuaally 3rd or 4th generation drugs, are used in a desperate attempt to control the infection which is essentially of unknown eitiology.

Although the use of antimicrobial drugs can lead to the resolution of infection in some cases of microbial keratitis, use of multiple of drugs can lead to the organisms becoming drug resistant.(77).

Severe infections and drug resistance pose a great challenge to the clinician in managing infectious keratitis.(80)

Thus, there is a need for newer, more general methods, that are safer and effective adjunctive to antimicrobials in the treatment of infective keratitis. These methods should be easily accessible, easily done, and effective against a wide range of organisms. This would eliminate the absolute requirements of microbiological smears, cultures and sensitivity testing, without increasing the risk of drug sensitivity.

Collagen Cross-linking is a procedure that shows some promise in this respect.

In the eye, the CXL technique using riboflavin and UV-A has wide-spread use for its cross-linking effect, which results in a stiffening effect, in the prevention of progression of keratoconus. In patients with keratoconus, this cross-linking effect has been found to occur upto a maximum depth of

300µ. Studies have shown that UV-A light penetration upto the endothelial level results in endothelial cell damage. UVA-light and Riboflavin-mediated

Corneal Collagen Cross-linking.(81) Additionally, crystalline lens damage may occur with exposure to UV-A light. Hence, in these patients, it is suggested that corneas less than 400µ thick not be treated with CXL as this may compromise the endothelium.4,5

This consideration in patients with corneal ulcer may differ from patients with keratoconus, who have clear, transparent corneas. The cornea around a corneal ulcer is thickened due to corneal edema. The area of the ulcer itself is hazy or opaque because of the infiltration with cells and organisms, as well as the fliud imbibition. This opacity itself would limit UV light penetration. Additionally, it is known that UV light penetration into transparent liquids are much more limited as compared to transparent solids.

Hence, in corneal ulcer patients the criteria of corneal thickness 400 microns or more may not be as critical because:

1. UV light penetration into the cornea is limited by the opacity caused by the ulcerated, edematous and moist corneal surface.

2. Crystalline lens damage leading to early cataract formatoion usually occur in patients with severe anterior segment inflamation, as in corneal ulcer patients. This is easily treated with a cataract surgery, either before, or during the corneal transplantation procedure.

3. After the corneal ulcer heals, there is always a scar necessitating a corneal transplant to regain vision. Hence endothelial damage (which may occur even just because of the ulcer itself), is not as relevant in this situation where it anyway will need to be removed for the corneal transplant.

In patients with superficial ulcers, the possibility of an anterior lamellar corneal graft exists. In this situation, the corneal endothelium has to be healthy, and therefore cannot be allowed to be compromised by the CXL procedure.

It is for this reason that we, in our study decided to put a limit of less than 50% thinning for patients to be eligible for collagen cross-linking.

As discussed in the literature review, there are several in vitro and in vivo studies demonstrating the antimicrobial efficacy of riboflavin and UV light.

In transfusion medicine, the riboflavin/UV-A treatment has extremely good safety profile for the blood components and is thus safe to use for preventing bacterial, viral and parasitic infections.17-22(Panda Article).

In vitro studies and in vivo studies have shown beneficial effects for several bacterial and fungal organisms including Staphylococcus aureus, methicillin resistant Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus pneumonia, drug resistant Pseudomonas aeruginosa, aspergillous and fusarium. (REF) Other susceptible organisms are Mycobacteriumchelonae, Non-tubercular mycobacterium, E. Coli and Acremonium. (REFS)However, there are mixed reports regarding the efficacy observed on Candida albicans, some reporting favourable responses, and others not. (REF).

With regard to the protozoan parasite, acanthamoeba, only one case report was found describing a favourable effect.(Yasin Khan) This was an in-vivo case report of three ulcers, two of which had microbiologically proven acamthamoeba, while the third was highly presumptive of it. These ulcers were unresponsive to conventional treatment, but were reported to dramatically improve following CXL. However, there were more reports that specifically found a poor outcome in acanthamoeba corneal ulcers. (REF)

Some of the studies reported better outcome with increased duration of UV-A exposure (60 minutes instead of 30 minutes). Hence, results of CXL in patients with Acanthamoeba corneal ulcers seems to be questionable, and inconclusive.

Viral corneal ulcers were also found to be unresponsive to CXL, and in some cases, re activation of viral keratitis was reported. (REFS)

Hence, for our study, we decided to exclude those patients with proven or suspected Acanthamoeba corneal ulcers, and patients with suspected active, or past history of treatment of viral keratitis.

In almost all the case reports, only one sitting of CXL was performed. We however felt that a larger number of exposures in recalcitrant ulcers may be more beneficial. We therefore treated our patients with a maximun of four exposures, at intervals of 48 hours. Long-term studies will be required to determine if this affects the outcome in an adverse manner e.g. increased rates of delayed vascularization. In our study, increased vascularization was not found to occur in any of the patients at the time of healng of the ulcer.

It is interesting to note that many studies report an almost dramatic symptomatic relief in patients following CXL even within 24 hours of the procedure. This could be because of the action of riboflavin/UVA on the nociceptive response of corneal nerves that decrease pain(82) (Bertollo 2006).

In our study, almost all patients did report feeling better, but this may have been a psychological effect of a “new” procedure with blue lights, done in the operation theatre, with all the attendant new experiences. One patient reported having significantly more pain on the day following the UV light

exposure. However, we cannot comment on these findings as our study was not designed to determine the patients' pain relief scale in an objective manner.

The number of patients in the prospective arm was too small to adequately comment on the reduction of "failure of treatment" rates. Out of the 11 patients who reached the end point of our study, one patient perforated, while the other 10 healed well without incident.

The patient who perforated had a deep stromal fungal ulcer (Fusarium), and had undergone intracameral amphoterecin injections as part of the standard treatment procedures. Other authors have described poor response to CXL in patients with deep stromal ulcers, and postulate that the UV-A light penetration may just not be adequate enough for a beneficial effect to occur. This was our experience as well. All the other ten patients had a more superficial ulcer, which was probably the reason they responded well to the CXL.

The biochemical changes that occur with cross-linking a cornea have been experimentally demonstrated and reported by several authors.(64) The normal ageing cornea undergoes cross-linking, and this effect is readily appreciated while doing a corneal transplantation. A donor cornea from a young child is much more difficult to handle because of its extreme flexibility. However, a donor cornea from and older person is much easier to handle.

This cross-linking effect is speeded up, and enhanced artificially during the CXL procedure. This has been experimentally demonstrated to actually reduce the enzymatic effect of collagen digesting enzymes produced by

1. micro-organisms causing the corneal ulcer

2. the inflamatory cells that are present in the ulcer as a response to the infection9.

The authors found that the digestion time of pepsin, trypsin and bacterial collagenase doubled after crosslinking the corneas with riboflavin and UVA. They were thus proved that this treatment has both biochemical as well as biomechanical effects.

Hence, there is ample evidence demonstrating a potential benefial effect of CXL in treatment of our patients who have suppurative corneal ulcers.

To summarize, the mechanism of action of riboflavin/UV-A is multifactorial as reported in the literature.

1. Riboflavin/UVA directly acting on the microbial DNA to damage it.
2. Collagen crosslinking strengthens the stromal collagen fibrils and decreases their enzymatic degradation.
3. CXL increases the tensile strength of stromal collagen.
4. Corneal apoptosis induced by CXL restores normal architecture of cornea.
5. CXL chemically alters the nucleic acid of the bacteria, reducing replication.
6. CXL reduces the inflammatory and immune cells, and
7. CXL reduces the nociceptive response of corneal nerves that decrease pain.

The present study was conducted on the basis of a retrospective analysis of corneal ulcer cases presenting to the Department of Ophthalmology, Christian Medical College& Hospital, Schell Campus, Vellore for the past one year (April 2013 to April 2014). In spite of complete microbiological investigations, and adequate standard antimicrobial therapy the healing time for our suppurative corneal ulcers was very prolonged; on an average 48.79 days (Range 7 to 151 days). The requirement of the patients to stay in the hospital for a long duration added to the cost of treatment because of the inability of these patients to go to work. Additionally, an extra relative that had to stay in the hospital along with the patient created an economic burden both for the patients and for the health provider. We also observed a "failure of treatment" rate of about 12.5%.

We decided to do this study to see if we could reduce the duration of hospital stay by reducing the time to healing, and also if we could reduce the "failure of treatment" rates even further.

Thus, in this prospective study conducted in our department , thirteen patients (7 males and 6 females) with suppurative ulcers size averaging 3.65mm x 3.57mm (range 1.1mm x 1.0mm to 6mm x 6mm) were consented and enrolled. This number was less than the calculated sample size of sixteen, as only thirteen patients who fit all the inclusion and exclusion criteria presented to us during the study period. Of these 13 patients, only eleven patients could be analysed as two patients had to be excluded because

1. One patient was lost to follow-up after discharge from the ward, and so healing could not be determined.

2. One patient is still undergoing treatment and had not healed at the end of the study period.

We however did find a substantial reduction in the healing time in ten out of the eleven patients. The ulcer healing time was in the CXL group was about half that of the group who had not received CXL - 21.6 days as compared to 48.79 days in the retrospective group. This reduction was not statistically significant (P=0.06), but is definitely greatly clinically significant. Reducing the healing time of a corneal ulcer would be a major reduction of the economic burden on our patients.

This evidence is in accordance to previously published clinical evidence on reduced healing time using CXL treatment as compared to antimicrobial drug treatment alone.(65,71,77,80,83)

We are however unable to comment on the small reduction of "failure of treatment seen here (1 out of 11 patients, or 9%) as the numbers in our prospective group were too small. We will however be continuing enrollment of patient after the duration of this study to determine if there is actually a reduction in the failure rates (perforation rates), with CXL, due to its property of increasing the resistance to enzymatic digestion of corneal collagen fibrils.

The efficacy of CXL does not apparently depend on the specific causative organism. The treatment works for several types of bacterial as well as fungal ulcers. In this respect, it is a "broad-spectrum" treatment. This could be an advantage in situations where microbiological investigations are too expensive for the patients, or not adequately available for use. CXL may help improve the outcomes in these situations.

Additional interesting facts that came out of this study are presented as follows.

There was a higher percentage of fungal keratitis patients in both the retrospective arms as well as the prospective arm (Figs.3 and 4) compared to bacterial ulcers. This is in keeping with published data regarding eitiology of corneal ulcers in South India as compared to data from the developed countries.

It is interesting to note the difference in the percentage of diabetics in the retrospective and the prospective arms of the study (Figs. 7 and 8). There were more diabetics found in the prospective arm (31%) as compared to the retrospective arm (16%) . This may of course be a reflection of the smaller sample size of the prospective group. However, in both groups, both the controlled as well as the uncontrolled diabetics (at admission) responded to the treatment given. However, in both groups, healing time was longer in the uncontrolled diabetics as compared to the controlled diabetics, a result that is to be expected.

The number of diabetics in each goup was too small to do any meaningful statistical analysis, but just eyeballing the data shows that the diabetics have a trend to healing faster with CXL. This will need to be further studied with a larger number of patients.

One of the concerns however, is the additional cost as well as the ready availability of the riboflavin. The good news is that Indian companies are currently marketing “Riboflavin” in various brand names, so as to make it more readily available.

We feel that the cost is offset by the reduction of the “time to healing”. Additionally, as treatment is done in an aseptic manner at 48 hour intervals, we found it was possible and safe to use a single vial of the riboflavin for at least two sittings of CXL, storing the remainder in the fridge under sterile conditions.

From this observational study with the number of patients we were able to recruit, we have found that the CXL technique does have substantial benefits for our patients and can be used for improving the outcome and reducing the time taken for healing suppurative corneal ulcers. However, we will need to study a larger number of patients before we can introduce this additional modality of treatment to our Standard Operating Procedure.

SUMMARY

SUMMARY

Infectious keratitis is a serious sight threatening problem world-wide, so it is considered a public health problem. Corneal ulcer is an ocular emergency. Most of the corneal ulcers need microbiological investigation, protracted treatment, longer stay in the hospital and longer healing time for adequate control. Corneal collagen crosslinking is a standard therapy for keratoconus to strengthen the corneal lamellae. The beneficial effect of corneal collagen cross-linking on corneal ulcers is still under evaluation.

The present study was undertaken to compare the effects of corneal collagen cross-linking on the healing time of corneal ulcers. 13 patients with corneal ulcers presenting to the ophthalmology outpatient department fitting in the selection criteria were included in the study. All patients after preliminary examination were put on topical antimicrobials. All patients underwent standard UV-A/Riboflavin cross linking within 48 hours. Corneal collagen cross-linking was performed up to a maximum four sessions with an interval of 48 hours. Relief of symptoms and time to healing was noted.

This result was compared retrospectively to healing time of corneal ulcers of patients who were admitted in the ward one year earlier, who had not undergone corneal collagen cross-linking. The data was taken from the case records.

The ulcers in the prospective arm had an average healing time of 21.6 days while the retrospective arm had an average healing time was 48.79 days.

This reduction was not statistically significant (P=0.06), but is definitely greatly clinically significant.

There was one failure of treatment, as one patient had corneal perforation.

Hence, we feel that CXL may be a viable option as an adjuvant therapy for microbial keratitis.

LIMITATIONS OF THE STUDY

LIMITATIONS OF THE STUDY

1. The determined sample size could not be achieved in the duration of this study.

2. The number of corneal collagen cross- linking procedures was not standardized and was determined by the evaluating Ophthalmologist.

3. The size of the ulcers studied was less than 6mm. These ulcers generally tend to heal earlier, with fewer complications.

4. Sample size is too small to develop a standard operating procedure.

5. Long term effect of Corneal collagen cross- linking treatment could not be observed in the time frame of our study

CONCLUSIONS

CONCLUSIONS:

1. CXL reduces the “time to healing” of suppurative corneal ulcers less than 6mm in diameter in our patient population..

2. CXL may not be beneficial for patients with predominantly ,deep stromal ulcers.

3. Use of CXL may help reduce “failure of treatment” of suppurative corneal ulcers..

4. Corneal vascularization may not be a major complication of CXL in the short term.

5. CXL may be used as an adjuvant to antimicrobial therapy.

6. Multiple CXL treatment schedules may be given in recalcitrant cases of suppurative corneal ulcers.

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BIBLIOGRAPHY

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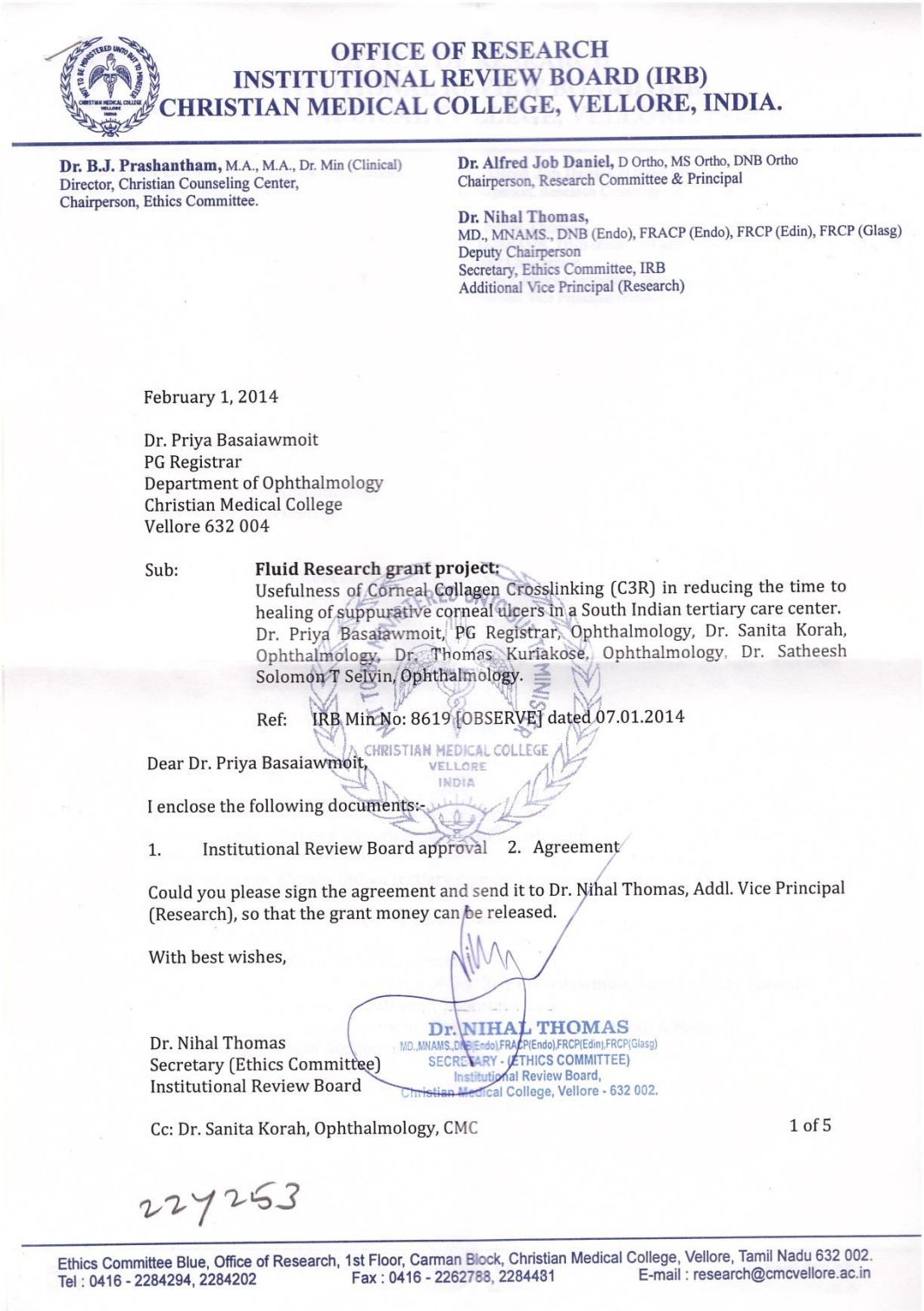
ANNEXURE

ANNEXURE I

IRB APPROVAL FORM



OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
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Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Alfred Job Daniel, D Ortho, MS Ortho, DNB Ortho
Chairperson, Research Committee & Principal

Dr. Nihal Thomas,
MD., MNAMS., DNB (Endo), FRACP (Endo), FRCP (Edin), FRCP (Glasg)
Deputy Chairperson
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

February 1, 2014

Dr. Priya Basaiawmoit
PG Registrar
Department of Ophthalmology
Christian Medical College
Vellore 632 004

Sub: **Fluid Research grant project:**
Usefulness of Corneal Collagen Crosslinking (C3R) in reducing the time to healing of suppurative corneal ulcers in a South Indian tertiary care center.
Dr. Priya Basaiawmoit, PG Registrar, Ophthalmology, Dr. Sanita Korah, Ophthalmology, Dr. Thomas Kuriakose, Ophthalmology, Dr. Satheesh Solomon T Selvin, Ophthalmology.

Ref: IRB Min No: 8619 [OBSERVE] dated 07.01.2014

CHRISTIAN MEDICAL COLLEGE VELLORE INDIA

Dear Dr. Priya Basaiawmoit,

I enclose the following documents:-

1. Institutional Review Board approval 2. Agreement

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

With best wishes,

Dr. NIHAL THOMAS
MD.,MNAMS.,DNB(Endo),FRACP(Endo),FRCP(Edin),FRCP(Glasg)
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board

Cc: Dr. Sanita Korah, Ophthalmology, CMC

1 of 5

224253

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002.
Tel : 0416 - 2284294, 2284202 Fax : 0416 - 2262788, 2284481 E-mail : research@cmcvellore.ac.in

ANNEXURE I

IRB APPROVAL FORM

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

B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
tor, Christian Counseling Center,
person, Ethics Committee.

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February 1, 2014

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PG Registrar
Department of Ophthalmology
Christian Medical College
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Ref: IRB Min No: 8619 [OBSERVE] dated 07.01.2014

Dear Dr. Priya Basaiawmoit,

The Institutional Review Board (Blue, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project entitled "Usefulness of Corneal Collagen Crosslinking (C3R) in reducing the time to healing of suppurative corneal ulcers in a South Indian tertiary care center." on January 7th 2014.

The Committees reviewed the following documents:

1. IRB Application format
2. Curriculum Vitae's of Drs. Priya Basaiawmoit, Sanita Korah, Thomas Kuriakose, Satheesh Solomon T Selvin.
3. Informed Consent (English, Tamil, Telugu, Hindi & Bengali)
4. No of documents 1-3

Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002.
16 - 2284294, 2284202 Fax : 0416 - 2262788, 2284481 E-mail : research@cmcvellore.ac.in

ANNEXURE I

IRB APPROVAL FORM

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

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Dr. Nihal Thomas,
MD., MNAMS., DNB (Endo), FRACP (Endo), FRCP (Edin), FRCP (Glasg)
Deputy Chairperson
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

The following Institutional Review Board (Blue, Research & Ethics Committee) members were present at the meeting held on January 7th 2014in the CREST/SACN Conference Room, Christian Medical College, Bagayam, Vellore 632002.

Name	Qualification	Designation	Other Affiliations
Dr. T. Balamugesh	MBBS, MD(Int Med), DM, FCCP (USA)	Professor, Pulmonary Medicine, CMCH.	Internal, Clinician
Dr. Mathew Joseph	MBBS, MCH	Professor, Neurosurgery, CMCH.	Internal, Clinician
Dr. J. Visalakshi	MPH, PhD	Lecturer, Dept. of Biostatistics, CMC.	Internal, Statistician
Dr. Susanne Abraham	MBBS, MD	Professor, Dermatology, Venerlogy & Leprosy, CMCH.	Internal, Clinician
Dr. Ranjith K Moorthy	MBBS M Ch	Professor, Neurological Sciences, CMCH.	Internal, Clinician
Dr. Vivek Mathew	MD (Gen. Med.) D.M (Neuro) Dip. NB (Neuro)	Professor, Neurology, CMC	Internal, Clinician
Mrs. Shirley David	M.Sc, PhD	Professor, Head of Fundamentals Nursing Department, CMCH	Internal, Nurse
Mrs. Pattabiraman	B. Sc, DSSA	Social Worker, Vellore	External, Lay person
Mr. C. Sampath	B. Sc, BL	Legal Expert, Vellore	External, Legal Expert
Dr. Ebenezer Ellen Benjamin	M.Sc, PhD	Professor, Maternity Nursing, CMCH.	Internal, Nurse

IRB Min No: 8619 [OBSERVE] dated 07.01.2014 3 of 5

ANNEXURE I

IRB APROVAL FORM

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

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
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Dr. Nihal Thomas,
MD., MNAMS., DNB (Endo), FRACP (Endo), FRCP (Edin), FRCP (Glasg
Deputy Chairperson
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

Dr. B. J. Prashantham	MA(Counseling Psychology), MA(Theology), Dr. Min(Clinical Counselling)	Chairperson, Ethics Committee, IRB. Director, Christian Counseling Centre, Vellore	External, Social Scientist
Dr. Jayaprakash Muliyil	B. Sc, MBBS, MD, MPH, Dr PH (Epid), DMHC	Retired Professor, Vellore	External, Scientist & Epidemiologist
Dr. Denise H. Fleming	B. Sc (Hons), PhD	Honorary Professor, Clinical Pharmacology, CMCH.	Internal, Scientist & Pharmacologist
Rev. Joseph Devaraj	B. Sc, BD	Chaplaincy Department, CMCH.	Internal, Social Scientist
Dr. Nihal Thomas,	MD, MNAMS, DNB(Endo), FRACP(Endo) FRCP(Edin) FRCP (Glasg)	Professor & Head, Endocrinology. Additional Vice Principal (Research), CMCH. Deputy Chairperson, IRB, Member Secretary (Ethics Committee), IRB	Internal, Clinician

We approve the project to be conducted as presented.

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.**

IRB Min No: 8619 [OBSERVE] dated 07.01.2014 4 of 5

ANNEXURE I

IRB APROVAL FORM

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

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Alfred Job Daniel, D Ortho, MS Ortho, DNB Ortho
Chairperson, Research Committee & Principal

Dr. Nihal Thomas,
MD., MNAMS., DNB (Endo), FRACP (Endo), FRCP (Edin), FRCP (Glasg)
Deputy Chairperson
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

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.

Fluid Grant Allocation:

A sum of 37,500 INR (Rupees Thirty Seven Thousand Five Hundred only) will be granted for 9 months.

Yours sincerely

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board

NOT TO BE MINISTERED UNTO BUT TO MINISTER
CHRISTIAN MEDICAL COLLEGE
VELLORE
INDIA

DR. NIHAL THOMAS
MD.,MNAMS.,DNB(Endo),FRACP(Endo),FRCP(Edin),FRCP(Glasg)
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

Cc: Dr. Sanita Korah, Ophthalmology, CMC

IRB Min No: 8619 [OBSERVE] dated 07.01.2014

5 of 5

ANNEXURE II

INFORMATION SHEET

Christian Medical College, Vellore

Department of Ophthalmology

Corneal collagen cross linking in corneal ulcers

Information sheet

You are being requested to participate in a study todetermine if adjunctive treatment with Ultra violet light in the presence of a photosensitising dye (a procedure called Collagen Cross-linking) will help stop the progression of corneal ulcers.

The procedure for Collagen Crosslinking involves exposureto a specific amount of Ultraviolet light onto the cornea for 30 minutes. The cornea is bathed in a special photosensitising dye (Riboflavin dye), that helps the UV light to penetrate deep enough. This procedure has been found to be very safe and free fromside-effects.

This procedure may be repeated up to a maximum of four times during the duration of this trial.

You will undergo collagen cross linking in addition to standard therapy. Two days after your hospital admission, if found suitable you will be requested to enrol. Then you will be started on the above mentioned procedure.

Clinical photographs of the eye will also be taken for documentation.

Your participation in this study is entirely voluntary and you are also free to decide to withdraw permission to participate in this study. If you do so, this will not affect your usual treatment at this hospital in any way.

The information gathered from this study will help us understand the usefulness of corneal collagen cross linking (C3R) in the management of corneal ulcers.

There will be no additional costsinvolved for you in the study. The procedure will be performed during the normal admission duration.

The results of this study will be published in a medical journal but you will not be identified by name in any publication or presentation of results. However, your medical notes may be reviewed by people associated with the study, without your additional permission, should you decide to participate in this study.

If you have any further questions, please ask Dr. Priya Basaiawmoit
(Tel: 0416 2281201 / 9944361981) or email: priya_doc18@yahoo.com

ANNEXURE III

CONSENT FORM

Informed Consent: Effect of Corneal Collagen Cross Linking in Corneal Ulcers

Study number: **Date:**

Name of participant:

Hospital number:

I confirm that I have been given the option of undergoing corneal collagen cross linking for my corneal ulcer within 48 hours of my admission.

The procedure of collagen cross linking has been explained to me in my own language and I have understood that this procedure may or may not have any benefit on my corneal ulcer. I have had the opportunity to ask the investigator any questions related to the procedure.

I understand that my participation in this study is voluntary and that I can leave the study at any given time, without having my medical care or legal rights being affected. I agree that the investigators and their team have the access to all the data that I may provide them. I accept to share the data obtained during analysis in the faith that it will be used only for scientific purposes. I accept that my identity will not be revealed if the data be published or sent to a third party. I agree not to restrict the scientific use of any of the data or results that may arise from this study.

Understanding all the above, I give my consent for taking part in the above mentioned study

Patient's signature (or thumb impression) / Legally acceptable representative's Signature (or thumb impression) with date

Signature of a witness with date

Signature of the investigator with date

ANNEXURE IV

PATIENT PROFILE

PATIENT PROFILE

Name: Hospital Number: Age:

Diagnosis(clinical): Eye:

Diabetes controlled /uncontrolled

Anaemia

Immunosupression HIV/HBV/HCV

Smear Report:

Fungal	Bacterial

Current therapy

Antifungal	Antibacterial

Other treatment

Glaucoma

POST C3R

Session 1

Parameters	Grading		
	-1	0	+1
Reduction of Pain			
Rounding of corneal infiltrates			
Reduction of height of hypopyon			
Reduction of size of epithelial defect			
A subjective “forced gut-feeling” of healing			
Total score			

Post C3R

Session 2

Parameters	Grading		
	-1	0	+1
Reduction of Pain			
Rounding of corneal infiltrates			
Reduction of height of hypopyon			
Reduction of size of epithelial defect			
A subjective “forced gut-feeling” of healing			

Post C3R

Session 3

Parameters	Grading		
	-1	0	+1
Reduction of Pain			
Rounding of corneal infiltrates			
Reduction of height of hypopyon			
Reduction of size of epithelial defect			
A subjective “forced gut-feeling” of healing			

Post C3R

4^{th} session

Parameters	Grading		
	-1	0	+1
Reduction of Pain			
Rounding of corneal infiltrates			
Reduction of height 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			