



Original Research Article

Benefit of collagen cross linking of the donor corneal button and the graft host junction during therapeutic penetrating keratoplasty - A pilot study

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ABSTRACT

Background: Collagen crosslinking (CXL), used as a treatment modality in infectious keratitis, can be further studied for the prevention of recurrent infection in cases undergoing therapeutic penetrating keratoplasty (TPK).

Purpose: To study the role of collagen crosslinking of the donor corneal tissue and the graft host junction during TPK in preventing recurrence of infection.

Materials and Methods: This was a prospective, clinical, interventional pilot case study done from May 2017 to July 2018. Fifteen cases and 15 controls who had infectious keratitis and needed TPK were included. Pediatric patients, one eyed patients, perforated ulcers and those with any other significant comorbidity were excluded. In patients who were enrolled as cases, the donor corneal graft and the graft host junction were subjected to CXL using modified Dresden protocol just after the TPK. TPK was done as per standard protocol. The patients were followed up for at least four months duration and the recurrences in both the groups were recorded.

Statistics: The Odds Ratio of the cases not developing a recurrent infection vis-a-vis the controls has been calculated along with the Confidence Interval using the SPSS Statistics software.

Results: Recurrence of infection was found in one case and in four controls within one month of TPK.

Conclusions: CXL done on donor corneal tissue and the graft host junction intra operatively, has a role in reducing recurrence of infection after TPK.

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1. Introduction

Therapeutic penetrating keratoplasty (TPK) is meant to terminate an actively infectious corneal disease and anatomically restore the cornea. Its primary goal is to eliminate infection and to re-establish globe integrity.¹ Corneal collagen crosslinking (CXL) treatment which is commonly performed to halt the progression of corneal ectasia, has recently also been used to treat infectious keratitis.¹⁻³ There are two effects of CXL on the cornea. Its biomechanical strengthening effect used in the treatment of corneal ectasia and in preventing melt of carrier donor cornea in Boston keratoprosthesis.⁴ Its strengthening as well as sterilizing effects are employed in photo activated

chromophore (PACK) CXL to treat corneal ulcers.⁵

The authors had done a study,⁶ hereafter referred to as TPK-CXL 1, to see the effect of CXL on donor corneal button just before doing TPK for infectious keratitis and had got encouraging results. Recurrence of infection after TPK in which the donor graft had undergone CXL was lesser as compared to controls.^{6,7}

So, extending along the same lines, CXL was performed intraoperatively so that along with the donor corneal button, the graft host junction was also exposed to CXL (hereafter referred to as TPK-CXL 2). Recurrence usually occurs from the recipient bed. So it was thought that if the recipient corneal rim also undergoes CXL, then recurrence rate might still reduce.

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This pilot study aims to determine the efficacy of corneal collagen cross-linking in preventing the recurrence of infection in microbial keratitis, when performed concurrently during therapeutic penetrating keratoplasty.

TPK-CXL 1⁶ used the strengthening effect of CXL on the donor cornea to retard recurrently invading organisms after therapeutic penetrating keratoplasty. TPK-CXL 2 employs the strengthening effect of CXL on the donor cornea as well as its sterilizing effect on the graft-host junction to prevent recurrent infection.

2. Materials and Methods

This study was a prospective, clinical, interventional pilot case study done at a tertiary eye care hospital from May 2017 to July 2018 after obtaining clearance from the Institutional review board and ethical committee. The study was conducted according to the guidelines of the declaration of helsinki. Fifteen cases and 15 controls selected randomly were enrolled in the study. Written informed consent was taken from all the patients who participated in the study.

2.1. Inclusion criteria

Microbiologically proven bacterial, fungal, viral, protozoal or mixed infectious keratitis and culture negative infectious keratitis requiring TPK were included in the study.

2.2. Indications for TPK

2.2.1. Corneal ulcers

1. Worsening despite maximum medical treatment
2. Extending to the limbus
3. Impending corneal perforation

2.3. Exclusion criteria

1. Age less than 18 years
2. Single eyed patients
3. Perforated corneal ulcers
4. Presence of significant systemic co-morbidities like immunosuppression
5. Presence of significant ocular co-morbidities like lagophthalmos, glaucoma, retinal detachment, etc.

A detailed medical history was taken for each patient to identify ocular, systemic or occupational predisposing factors that may make the cornea susceptible for developing microbial keratitis. Documentation of the best corrected visual acuity (BCVA) and a comprehensive ocular examination consisting of slit-lamp biomicroscopic examination, digital tension for intraocular pressure and fundus examination if possible were done. Ultrasonography to evaluate the posterior segment was done if fundus examination was not possible.

Slit lamp bio microscopy included an examination of the precorneal tear film, conjunctiva, cornea, anterior chamber,

iris, lens and anterior vitreous. The following features of the corneal ulcer were recorded carefully:

1. Location of the corneal ulcer- Central, paracentral, peripheral (within 3 mm of limbus) or total.
2. Shape of the ulcer
3. Margins of the ulcer-The margins or the edges of the ulcer varied according to the cause and stage of the ulcer.
4. Size of the ulcer- Size of the ulcer was recorded with the help of the slit lamp micrometer.
5. Epithelial defect- The epithelial defects were stained with fluorescein dye and the size measured in the two largest meridians with the help of slit-lamp micrometer.
6. Infiltrates- In case of multiple infiltrates, each one was documented separately. The infiltrate was measured in the two largest dimensions and recorded on a schematic corneal diagram, particularly in relation to the limbus.
7. Corneal vascularisation- A quadrant wise record of corneal vascularization was made.
8. Corneal sensation- This was checked with a sterile cotton wisp.
9. Corneal thinning / perforation- In the presence of shallow anterior chamber and low intraocular pressure, a Seidel's test was performed.
10. Anterior chamber reaction-The anterior chamber reaction ranged from mild flare and cells to severe hypopyon formation. A record of the size of the hypopyon using the slit-lamp micrometer and its characteristics were made.

2.4. Documentation

Documentation of the size and features of ulcer was done by clinical digital slit lamp photography and by using color-coded diagrams. At all visits, digital photographs of the diffuse as well as the slit section of the corneal ulcer were taken and drawings of the corneal ulcer along with the surrounding cornea were made using a standard scheme of color-coding and shading.^{8,9}

2.5. Investigations

All included patients were subjected at presentation, to a set of ocular and systemic investigations including microbiological investigations integral to the workup of a case of infectious keratitis. Treatment was initiated based on the results of smear examination and if required, modified in accordance with the culture and sensitivity results and the clinical course. The most important sample for microbiological examination was the corneal scraping.¹⁰ Samples were also obtained from the contact lenses, contact lens case and contact lens solutions if the patient was a contact lens user.

Culture on standard media was the gold standard for the diagnosis of microbial keratitis. The corneal scrapings were routinely inoculated onto blood agar plate, chocolate agar plate, Sabouraud's dextrose agar and anaerobic media.

Patients who steadily worsened on medical therapy underwent therapeutic penetrating keratoplasty. Intraoperative CXL was also performed for the cases whereas the controls underwent only TPK.

2.6. The TPK procedure

1. All therapeutic penetrating keratoplasty procedures were performed under local or general anesthesia, as per standard protocol after clearance from a physician and an anaesthetist.
2. After adequate exposure of the eye, the host trephine was selected to cover the infiltrate edge of the ulcer completely with a 2mm margin of healthy host tissue wherever possible.
3. The donor button was oversized by 0.5mm-1mm and punched from the endothelial side of the corneoscleral button. Maximum graft diameter that was needed was 10.5 mm.
4. The host button was trephined and removed with the use of corneal scissors.
5. Purulent and fibrinous material was washed off from the anterior chamber.
6. Thorough irrigation was done to ensure thorough cleaning of the anterior chamber and angle of any exudates and release any peripheral anterior synechiae.
7. Cataract if found was not disturbed unless the anterior lens capsule was breached.
8. One or more peripheral iridectomies were performed to avoid post operative pupillary block.
9. The donor button was sutured to the host with 20-24 interrupted 10-0 nylon sutures depending on the graft size.
10. Viscoelastic substance was completely washed off from anterior chamber.
11. Suture knots were trimmed and buried on the donor side.
12. The wound was checked meticulously for absence of leakage.
13. Intracameral antibiotics/antifungals were injected as required. The choice of antimicrobial depended on the causative organisms.

2.7. The CXL procedure: (modified dresden protocol)¹¹

1. After the completion of TPK, corneal collagen crosslinking was performed after measuring the area of any pre existing epithelial defect or disturbance in the donor graft.
2. 0.1% Riboflavin solution in 20% dextran was then applied over the cornea every 2-5 minutes, starting

30 minutes before UVA exposure to allow stromal saturation. The irradiation was performed from a distance of 5 cm for 30 minutes.

3. A UVA diode at a wave length of 365-370nm was used to deliver an irradiance of 3mW/cm²- a total dose of 5.4 J/cm² of the cornea.^{12,13}
4. Ultraviolet beam diameter of 11mm was used to cover the graft host junction to the maximum extent possible. A limbal protection ring was used to protect limbal stem cell damage.¹⁴
5. The annulus of the graft host junction within the limbal protection ring was subjected to the CXL procedure along with the donor graft.
6. Our previous study⁶ used only the strengthening effect of CXL on the donor cornea. This study attempts to utilize:
 7. The strengthening effect on the donor cornea
 8. Sterilizing effect at the graft host junction
 9. Possible antimicrobial property of Riboflavin to prevent recurrent infection in the graft.

After the surgery, the host corneal button and the donor corneoscleral rim were sent to microbiology for Gram's/KOH staining, bacterial and fungal culture and histopathology.

After the surgery, topical antimicrobials were started depending on the microbiology reports. All cases and controls were assessed on the 1st postoperative day, 3rd post-operative day, 1st postoperative week every week till one month post operatively and thereafter every month for three months and then three monthly for one year and thereafter, annually.

All the patients underwent a complete ophthalmic examination including a detailed slit lamp evaluation at all the post-treatment follow-ups with special attention to the following features.

1. Signs of recurrent infection
2. Graft clarity
3. Graft host junction health
4. Epithelial integrity
5. Suture assessment for exposed suture knots, loose sutures, infiltrates
6. Corneal vascularisation
7. Peripheral anterior synechiae
8. Anterior chamber reaction

The observations were documented by the same corneal clinician so as to minimize the inter-observer bias. Findings were documented using schematic drawings and coloured photographs before and after the procedure. Fifteen patients meeting the previously mentioned inclusion and exclusion criteria, who underwent therapeutic penetrating keratoplasty without intraoperative CXL in the same period, served as the controls for the study.

Outcome measures of the study were:

1. Cure of infectious disease after surgery
2. Epithelial healing/anatomical integrity
3. Graft clarity

Infections were considered cured if there was no evidence of recurrent corneal infiltration suggestive of infection for one month after the keratoplasty. Anatomical success was considered if the integrity of the eye was restored.

3. Results

This was a prospective, clinical, interventional pilot case study with 15 cases and 15 controls done during period of May 2017 to July 2018. Mean age of cases was 46.33 ± 10.98 years (Range 26-70 years) and of controls was 50.46 ± 13.47 years (Range 20-77 years).(Table 1)

Table 1: Demographic characteristics of cases and controls

Characteristic	Cases (N=15)	Controls (N=15)
Age group (in years)		
≤20	0	1
21-30	1	0
31-40	3	2
41-50	6	4
51-60	4	5
61-70	1	2
>70	0	1
Sex		
Male	9	10
Female	6	5
Eye affected		
Right Eye	2	8
Left Eye	13	7

History of trauma/foreign body (FB) fall in eye and presence of any additional risk factors are shown in Table 2.

Table 2:

	Cases N=15	Controls N=15
History of trauma/foreign body fall		
Present	3	4
Absent	12	11
History of Diabetes mellitus		
Present	3	2
Absent	12	13

Etiology was fungal in 13 cases and two cases were culture and smear negative. Among controls, etiology was fungal in 11, mixed bacterial and fungal in one, Acanthamoeba in one, Microsporidial in one and, one control was culture and smear negative. Microbiological investigations and their results are shown in Table 3.

Table 3:

	Cases (N=15)	Controls (N=15)
Corneal scraping		
Positive	8	13
Negative	7	2
Corneal button culture		
Positive	13	14
Negative	2	1
Type of organism		
Fungal	13	11
Bacterial	0	0
Mixed	0	1
Acanthamoeba	0	1
Microsporidia	0	1

All surgeries were uneventful except two cases and two controls in whom auto expulsion of lens occurred due to severe upthrust and anterior vitrectomy was done. These are shown in Table 4.

Table 4:

	Cases N=15	Controls N=15
Uneventful	13	13
Eventful (severe upthrust)	2	2

3.1. Details of recurrence

3.1.1. Recurrence among cases and management

Recurrence of infection was found in one case. The primary etiology was fungal and recurrence also occurred with the same organism at one week follow up. The case with recurrence needed Re-TPK.

3.1.2. Recurrence among controls and management

Recurrence of infection was found in four controls. Of those, three were having primary etiology as fungal and recurrence also occurred with the same organism whereas in one patient, primary etiology was fungal but recurrence occurred due to bacteria. Three controls with recurrence needed repeat TPK whereas, one patient had extensive extension of infection to posterior segment and the eye needed to be eviscerated.

There was recurrence in one case and four controls among 15 cases and 15 controls. The duration of recurrence ranged from the 4th post-operative day to 25 days after the TPK. These are shown in Table 5.

The odds ratio (OR) is 5.09 [Confidence interval (CI) of 95% (0.5, 52.29)] and it shows a statistically positive association between CXL done on donor corneal tissue and the graft host junction and reduction in recurrence of infection following the procedure. The 'p' value was not calculated in view of the small sample size. Further studies

Table 5:

	Recurrence	Details of recurrence			Management done for recurrence
		Primary etiology	Duration between surgery and recurrence	Organism responsible for recurrence	
Cases N=15	1	Fusarium	6 days	Fusarium	Re- TPK
		Aspergillus flavus	5 days	Aspergillus flavus	Re-TPK
Controls N=15	4	Penicillium	14 days	Penicillium	Evisceration
		Fungus (no identification)	25 days	Gram Positive Cocci (no identification)	Re-TPK
		Yeast	4 days	Yeast	Re-TPK

to prove the role of CXL intraoperatively during TPK in preventing recurrent infection would be useful as the Odds Ratio is positive and within the 95% confidence interval in this pilot study.

Post-operative secondary glaucoma developed in two cases and three controls. Additional procedures in the form of re suturing of the graft was done in one case, Ahmed glaucoma valve surgery was done in one case and one case needed intracameral and intravitreal antibiotic injections. Whereas amongst controls, one required resuturing, three required intravitreal injections and two required tarsorrhaphy for persistent epithelial defect. These are shown in Table 6.

There was no adverse event due to the intra operative CXL.

Patients were followed up for minimum four months duration. Topical steroids were started for clear grafts with healed epithelium and without recurrence at one month post-operative follow up. Pre-operative and post-operative best corrected visual acuity (BCVA) are shown in Table 7.

At the time of last follow up, four grafts were clear and 11 failed amongst cases; whereas in controls, three grafts were clear, 11 grafts failed and one was eviscerated. These are shown in Table 8.

4. Discussion

Severe infectious keratitis often needs therapeutic penetrating keratoplasty and recurrence of infection after TPK can compromise the goal of the surgery. Infective hypopyon, anterior chamber exudates, perforated corneal ulcer, corneal infection extending to limbus, lens infection and posterior segment involvement are major risk factors for recurrence of infection after TPK.¹⁵ Recurrence of infection can present as contiguous infection from the recipient bed to graft, anterior chamber recurrence in the form of exudates and rarely infection in the posterior segment in the form of endophthalmitis.¹⁵ Recurrent infection results in poor anatomical and functional outcome of the TPK.

The success of CXL as a modality to modify the clinical course in keratoconus fueled alternative applications of

CXL in other corneal diseases as well. CXL done according to the modified Dresden protocol using ultraviolet A (UV-A) and riboflavin is a treatment that was developed to increase the biomechanical strength of cornea and halt the progression of keratoconus.¹¹ The procedure is based on using Riboflavin as a photosensitizer which generates reactive oxygen species when activated by UV-A at 370 nm. By way of photochemical reactions, these give rise to covalent bonds or cross-links in the corneal stroma.¹² It induces a change in the property of the collagen and has a stiffening effect on the corneal stroma which stabilizes it and increases its resistance to enzymatic degradation avoiding the progression of corneal melting.^{16,17} This is also utilized to prevent melting of the corneal graft used as a carrier in Boston keratoprosthesis.¹⁸ The photoactivation of riboflavin damages the RNA and DNA of microorganisms by oxidation processes causing lesions in their chromosomal strands.¹⁹ Riboflavin has a planar structure that intercalates between bases of DNA and RNA which results in oxidation of nucleic acids when irradiated by UV-A. This antimicrobial and strengthening effect of CXL has been utilized for treating infectious keratitis known as photo activated chromophore (PACK) CXL.⁵

Ours is a novel concept inspired by the use of CXL in the treatment of infectious keratitis and in the preoperative corneal collagen cross-linking of the graft tissue used as a carrier to Boston keratoprosthesis to decrease the risk of collagenolysis and corneal melting. In an earlier study, we attempted to utilize the biomechanical strengthening effect of CXL on the donor cornea before doing therapeutic keratoplasty. The results of prophylactic CXL on donor cornea in preventing recurrent infection after TPK were encouraging.

This concept was extended to intra operative CXL in this pilot study. This utilized the strengthening effect of CXL in the therapeutic graft as well as its sterilizing effect on the graft host junction. This reduced the recurrence due to any micro or macro amounts of infection left behind in the patient’s cornea.

Pre-operative pachymetry of the donor graft was not done as all the tissues had slightly swollen up while in the storage

Table 6:

	Cases N=15	Controls N=15
Post-operative rise in Intraocular pressure		
1. Present	2	3
2. Absent	13	12
Additional Procedures		
Re suturing	1	1
Glaucoma surgery	1	0
Intravitreal injection/ vitreo retinal surgery	1	3
Tarsorrhaphy	0	2

Table 7:

Cases		Controls	
Pre-operative BCVA	Post-operative BCVA	Pre-operative BCVA	Post-operative BCVA
PL+, PR accurate	6/12	PL+, PR accurate	PL+, PR accurate
6/36	6/18	CFCF	CFCF
6/24	HM+	PL+, PR accurate	HM+
PL+, PR accurate	PL+, PR accurate	2/60	HM+
PL+, PR accurate	PL+, PR accurate	CFCF	Eviscerated
HM+	PL+, PR accurate	PL+, PR accurate, HM+	PL+, PR accurate
HM+	CFCF	PL+, PR accurate	CF at 50 CM
PL+, PR accurate	PL+, PR inaccurate	HM+	PL+, PR accurate
CF at 2 meters	CF at 1 meter	PL+, PR accurate	CF at 1 METER
PL+, PR accurate	PL+, PR accurate	PL+, PR accurate	PL+
CFCF	CFCF	CFCF	PL+, PR accurate
CF at 2 meters	6/36	PL+, PR accurate	HM+
PL+, PR inaccurate	PL+, PR accurate	HM+	CF at 1 meter
PL+, PR inaccurate	PL+, PR accurate	6/12	6/18
PL+, PR accurate	3/60	CFCF	PL+, PR accurate

BCVA – Best corrected visual acuity, PL – perception of light, PR – projection of rays, CFCF – counting fingers close to face, CF – counting fingers.

Table 8:

	Cases	Controls
Clear	4	3
Failed	11	11

medium. Any pre-existing epithelial defect or disturbance in the donor graft was documented and was thought to allow soaking in of the Riboflavin dye. Further epithelial debridement was not done as that itself would be a risk for recurrent infection. A large diameter UV beam ensured irradiation of the graft host junction. A limbal ring was used to protect the limbal stem cells. The circular strip of the graft host junction inner to this ring was subjected to the CXL. The maximum diameter UV beam and the limbal protection ring are limitations that may preclude the use of this technique in very large corneo-scleral grafts more than 11 mm in diameter.

This pilot study shows reduced recurrence of graft infection in patients who underwent TPK with CXL. If confirmed with a larger randomized controlled trial, it will significantly reduce the chances of graft failure secondary to re-infection. This would be a novel method of successfully

treating microbial keratitis and would reduce the need for repeated surgeries, thereby saving valuable tissues and improve the anatomical outcome of therapeutic keratoplasty done for infectious keratitis.

5. Conclusion

CXL done on donor corneal tissue during transplantation to the donor button and graft host junction is safe. It is effective in reducing recurrence of infection after TPK for infectious keratitis.

6. Source of Funding

None.

7. Conflicts of Interest

The authors declare that there is no conflict of interest.

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