Safety and efficacy of collagen cross linking for donor corneal button in therapeutic penetrating keratoplasty

Kshama Popat[1,*], Radhika Natarajan[2]

[1]Senior Resident, [2]Deputy Director, Dept. of Cornea and Refractive Surgery, Medical Research Foundation, Sankara Nethralaya, Chennai, Tamil Nadu, India

***Corresponding Author: Kshama Popat**
Email: forgive_02@yahoo.com

Abstract
Purpose: To study the role of collagen crosslinking (CXL) of donor corneal tissue in preventing recurrence of infection after therapeutic penetrating keratoplasty (TPK).
Materials and Methods: This was a prospective, clinical, interventional case study done from May 2016 to July 2017. 15 cases and 15 controls were enrolled in the study. Patients who had infectious keratitis and needed TPK were included in the study. Pediatric patients, one eyed patients, perforated ulcers, patients with limbal/scleral involvement or posterior segment involvement or any other significant co-morbidity were excluded. In patients who were enrolled as cases, donor cornea was subjected to CXL using modified Dresden protocol just before TPK. TPK was done as per standard protocol. 3 cases and 2 controls had lens removal and required vitrectomy. All cases and controls were assessed post operatively on day 1, day 7 and 1 month post operatively. Digital slit lamp photos were taken at each visit for documentation of recurrence of infection, if any. The patients were followed up for at least 3 months duration.
Results: Etiology was fungal in 13 cases and 2 cases were culture and smear negative. Among controls, etiology was fungal in 10, mixed bacterial and fungal in 1, acanthamoeba in 2, microsporidial in 1 and 1 was culture and smear negative. Recurrence of infection was found in 2 cases and in 4 controls within 1 month of TPK (Odd's ratio 2.36).
Conclusion: CXL done on donor corneal tissue before transplantation has got a positive role in reducing recurrence of infection after TPK.

Keywords: Collagen crosslinking, Donor cornea, Infectious keratitis, Therapeutic penetrating keratoplasty.

Introduction

In developing countries, infective keratitis is one of the major causes of visual impairment.[1] Virulent organisms, lack of accessibility to eye care and delayed management may result in large or perforated corneal ulcer which may necessitate Therapeutic Penetrating Keratoplasty (TPK). TPK is meant to terminate an actively infectious corneal disease and anatomically repair the cornea. Its primary goal is to eliminate infection and to re-establish globe integrity.[2] TPK in advanced corneal ulcer carries a high chance of recurrence of infection which defeats the primary purpose staking structural integrity of the operated eye.[3] Corneal collagen cross-linking (CXL) was originally introduced as a treatment for corneal ectasia by Wollensak et al.[4] Recently it has been used in the treatment of infective keratitis and in the preoperative cross-linking of the graft tissue used as a carrier in Boston Keratoprosthesis to decrease risk of corneal melting due to collagenolysis and sterile keratolysis.[5-9]

There are two effects of CXL: sterilizing effect (used in infective keratitis) and biomechanical strengthening effect (used in treatment of corneal ectasia, infective keratitis and in preventing melt of carrier donor cornea in Boston Keratoprosthesis). Because of encouraging results of use of CXL in infective keratitis and in preventing corneal melts and enzymatic degradation in donor graft of Boston Keratoprosthesis, we hypothesized that application of prophylactic collagen cross linking (CXL) on donor cornea before transplantation might reduce chance of recurrence in therapeutic graft. It is the biomechanical strengthening effect of CXL which is being utilized here as strengthening helps to protect donor cornea against collagenolysis by recurrently invading organisms.

Materials and Methods

This study was prospective, clinical, interventional case study done at Medical Research Foundation from May 2016 to July 2017 after obtaining clearance from the Institutional Review Board and Ethical Committee. 15 cases and 15 controls were enrolled in study. Written informed consent was taken from all patients who participated in study.

Inclusion Criteria: Microbiologically proven bacterial, fungal, viral, protozoal or mixed infectious keratitis and culture negative infectious keratitis requiring TPK were included.

Indications of TPK

Corneal Ulcers

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

Exclusion Criteria:

1. Pediatric patients < 18 years age
2. Single eyed patients
3. Patients on immunosuppressants
4. Perforated corneal ulcers
5. Presence of other risk factors like lagophthalmos
6. Infections caused by atypical micro-organisms like *Pythium* species
7. Presence of anterior chamber exudates contiguous with corneal ulcer, other than hypopyon
8. Patients with scleral or posterior segment involvement
9. Uncontrolled diabetics

A detailed medical history was taken from each patient to identify ocular, systemic or occupational predisposing factor that may make cornea susceptible for developing microbial keratitis. A thorough general and adnexal

examination was performed to look for presence of facial palsy, abnormal blink rate, corneal/conjunctival exposure etc. Documentation of best corrected visual acuity (BCVA) of both eyes, slit-lamp biomicroscopic examination, digital tonometry for intraocular pressure (IOP) in affected eye and Goldmann's applanation tonometry (GAT) for other eye and fundus evaluation of both eyes was done. Ocular ultrasound of the affected eye was done if fundus examination was not possible. Lacrimal sac syringing was done for both eyes.

Slit lamp biomicroscopy included an examination of the precorneal tear film, conjunctiva, cornea, episclera, sclera, anterior chamber, iris, lens and anterior vitreous. The following features of corneal ulcer were recorded in detail:

Location: Central, paracentral, peripheral (within 3 mm of limbus) or total.

Shape

Margin: Well defined, ill-defined, feathery.

Size of Epithelial Defect: Measured in the two largest meridians after staining with fluorescein dye.

Infiltrates: Measured in the two largest dimensions particularly in relation to the limbus. In case of multiple infiltrates each one was documented separately. Size, colour and texture of infiltrates were noted.

Corneal Vascularisation: A quadrant-wise record of corneal vascularization was made

Corneal Sensation: Checked with a cotton wisp

Corneal Thinning/Perforation: In the presence of shallow anterior chamber and low intraocular pressure, a Seidel's test was performed to check for perforation

Anterior Chamber Reaction: In the form of flare and cells to severe hypopyon formation was recorded.

Documentation: Documentation of size and features of ulcer was done by using color-coded diagrams and digital slit lamp photographs in all visits.

Investigations: All patients were subjected to ocular and tailored systemic investigations including microbiological investigations. The treatment was initiated based on results of smear examination and modified in accordance with culture and sensitivity results. The most important sample taken for microbiological examination was corneal scraping.[10,11]

Culture was taken as gold standard for diagnosis of microbial keratitis. The corneal scrapings were inoculated onto blood agar, chocolate agar, Mac Conkey agar, Sabouraud's dextrose agar and brain heart infusion broth media.

Donor Corneal Button Preperation: Corneoscleral button preserved in Mc Carey Kaufman culture medium was mounted on an artificial anterior chamber. CXL was done according to modified Dresden protocol. A 0.1% riboflavin solution in 20% dextran was applied over the preserved corneoscleral button every 2–5 minutes for 30 minutes before ultraviolet A (UV-A) exposure to allow stromal saturation. The UV-A irradiation was given from a distance of 5 cm for 30 minutes. A UV-A diode at a wave length of 365–370 nm was used to deliver an irradiance of 3mW/cm^2 (a total dose of 5.4 J/cm^2). Riboflavin was applied every 5 minutes during UVA irradiation.

Surgical Procedure

1. All procedures were performed under local or general anesthesia after clearance from physician and anaesthesiologist.
2. Host trephine was selected to cover infiltrated edge of ulcer completely with a 2 mm margin of healthy host tissue wherever possible. Marking was made on the host cornea with selected trephine.
3. The crosslinked donor corneoscleral button 0.5-1mm larger than the host button was punched from endothelial side.
4. Host cornea was trephined and cut with Vannas scissors.
5. Purulent and fibrinous material was irrigated from the anterior chamber.
6. Visco dissection of angle was done to ensure thorough cleaning of the anterior chamber of any exudates and release any peripheral anterior synechiae.
7. Cataract if found was left undisturbed as much as possible.
8. In eyes with upthrust resulting in expulsion of lens, aphakia or vitreous loss, anterior vitrectomy was done.
9. The donor button was sutured to the host with 16, 20 or 24 interrupted sutures depending on the graft size.
10. Suture knots were trimmed and buried on donor side.
11. One or more peripheral iridectomies were performed to avoid postoperative pupillary block.
12. Anterior chamber was formed and the wound was checked at the end for any leakage.

The host corneal button and donor corneoscleral rim were sent to microbiology for Gram's staining, 10% KOH staining, bacterial culture and fungal culture and for histopathology.

Controls: In patients who were enrolled as controls, all steps were same except that donor cornea was not subjected to cross-linking before transplantation.

Follow Up: Postoperatively, topical antimicrobials were started depending on microbiology reports. Follow-ups were done on day 1, day 3, weekly for a month, and monthly for the first 3 months.

All patients underwent detailed slit lamp evaluation at all follow ups with special attention to any signs of recurrence of infection, epithelial integrity, exposed suture knots, loose sutures, infiltrates, graft edema, graft-host junction stability, corneal vascularisation, peripheral anterior synechiae and anterior chamber reaction. BCVA and IOP were noted at each visit.

Documentation was done by using schematic drawings and digital slit lamp photographs before and after TPK.

All patients were followed up for minimum 3 months after TPK.

Outcome Measure: Outcome was determined in terms of epithelial healing, recurrence of infection and graft clarity. Recurrence was diagnosed if there was a corneal infiltrate or hypopyon or posterior segment involvement within 1 month of TPK.[12]

Results

This was a prospective, clinical, interventional case study with 15 cases and 15 controls done during period of

May 2016 to July 2017. Demographic characteristics of cases and controls are shown in Table 1.

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).

History of trauma/foreign body (FB) in eye and presence or absence of diabetes mellitus (DM) systemically which could act as additional risk factor is shown in Table 2.

Microbiological etiology is shown in Table 3.

Table 1: Demographic characteristics of cases and control

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

Table 2: Additional risk factors

	Cases (n=15)	Controls (n=15)
History of Trauma/foreign Body Fall		
Present	3	4
Absent	6	8
History of DM		
Present	3	2
Absent	12	13

Table 3: Microbiological investigations and results

	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	2
Microsporidia	0	1

Details of Recurrence

Recurrence among Cases and their management: Recurrence of infection was found in 2 cases. 1 was having primary etiology as fungal and recurrence also occurred with same fungus where as in 1 patient, primary and recurrence causing organism couldn't be isolated. 1 case with recurrence needed Re-TPK while other case was managed with cyanoacrylate glue and bandage contact lens as the patient was not willing for surgery.

Recurrence among Controls and their management: Recurrence of infection was found in 4 controls. Of those, 3 were having primary etiology as fungal and recurrence also occurred with same fungus whereas in 1 patient, primary etiology was fungal but recurrence occurred due to bacteria. 3 controls with recurrence needed repeat TPK whereas 1 patient had extensive extension of infection extending to posterior segment and eye needed to be eviscerated.

The duration of recurrence ranged from the 4th post-operative day to 23 days after TPK. This is shown in Table 4.

There was recurrence in 2 cases and 4 controls in a sample of 15 cases and 15 controls viz. Odds ratio was 2.36 (Confidence interval of 95% (0.36,15.45)) and it shows statistically positive (significant) association between CXL done on donor cornea and reduction in recurrence following TPK. Same is shown in Table 5.

Post-operative secondary glaucoma developed in 4 cases and 3 controls.

There was no adverse event due to preoperative CXL.

Pre-operative and post-operative best corrected visual acuity (BCVA) are shown in Table 6.

At last follow up, 3 grafts were clear and 12 failed amongst cases, whereas in controls, 3 grafts were clear, 11 grafts failed and 1 was eviscerated.

Table 4: Details of recurrence

	Recurrence	Details of recurrence			
		Primary Etiology	**Duration between Surgery and Recurrence**	**Organism Responsible for Recurrence**	**Management done for Recurrence**
Cases (n=15)	2	Fusarium	6 days	Fusarium	Re- TPK
		Culture negative	23 days	Culture negative	Glue + BCL
Controls (n=15)	4	*Aspergillus flavus*	5 days	Aspergillus flavus	Re-TPK
		Penicillium	14 days	Penicillium	Evisceration
		Fungus (no identification)	20 days	Gram Positive Cocci (no identification)	Re-TPK
		Yeast	4 days	Yeast	Re-TPK

Table 5: Odds ratio calculation

	Non-recurrence	Recurrence	Total
Cases	13	2	15
Controls	11	4	15
Total	6	24	30

Odds ratio = ad/bc
= 13*4/2*11
= 52/22
Odds ratio = 2.36

Table 6: Comparison of pre-operative and post-operative BCVA

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

Discussion

Severe infectious keratitis needs TPK and recurrence of infection after TPK can compromise the aim of 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.[13] Recurrence of infection can present as contiguous infection from recipient bed to graft, anterior chamber recurrence in form of hypopyon or exudates and rarely infection in the posterior segment and vitreous in form of endophthalmitis.[13] Recurrent infection results in poor anatomical and functional outcome of the TPK.

Success of CXL in arresting progression of keratoconus fueled alternative applications of CXL in other corneal diseases. It 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 corneal stroma.[4] It induces a change in the property of collagen and has a stiffening effect on corneal stroma which stabilizes it and increases its resistance to enzymatic degradation avoiding progression of corneal melting.[15,16] This is also utilized to prevent melting of corneal graft used as a carrier in Boston Keratoprosthesis.[9] The photoactivation of riboflavin damages the RNA and DNA of micro-organisms by oxidation processes causing lesions in their chromosomal strands.[17] 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 antibacterial and strengthening effect of CXL has been utilized for treating infectious keratitis.[18]

Ours is novel concept which attempts to utilize biomechanical strengthening effect of CXL on the donor cornea. We got encouraging results of prophylactic CXL on donor cornea in preventing recurrent infection after TPK. To our knowledge, this is a pioneering study so comparison of results with literature was not possible.

This is a pilot study showing encouraging results proving that CXL is safe and effective in reducing recurrence following TPK and a randomized control trial with a large sample size can be done.

Conclusion

In conclusion, CXL done on donor corneal tissue before transplantation is safe. It is effective in reducing recurrence of infection after TPK for infectious keratitis.

Conflict of Interest: None.

Financial Interest: None.

References

1. Whitcher JP, Srinivasan M. Corneal ulceration in the developing world – a silent epidemic. *Br J Ophthalmol*. 1997;81(8):622–623.
2. Sharma N, Sachdev R, Jhanji V, Titiyal JS, Vajpayee RB. Therapeutic keratoplasty for microbial keratitis. *Curr Opin Ophthalmol*. 2010;21(4):293-300.
3. Bajrachrya L, Gurung R. Outcome of therapeutic penetrating keratoplasty in a tertiary eye care centre in Nepal. *Clin Ophthalmol* 2015;9:2299-2304.
4. Wollensak G, Spoerl E, Seiler T. Riboflavin/ultraviolet-A-induced collagen crosslinking for the treatment of keratoconus. *Am J Ophthalmol* 2003;135(5):620–627.
5. Al-Sabai N, Koppen C, Tassignon MJ. UVA/riboflavin crosslinking as treatment for corneal melting. *Bull Soc Belge Ophtalmol* 2010;(315):13–17.
6. Iseli HP, Thiel MA, Hafezi F, et al. Ultraviolet A/riboflavin corneal cross-linking for infectious keratitis associated with corneal melts. *Cornea* 2008;27(5):590–594.
7. Makdoumi K, Mortensen J, Crafoord S. Infectious keratitis treated with corneal crosslinking. *Cornea* 2010;29(12):1353–1358.
8. Schnitzler E, Spörl E, Seiler T. Irradiation of cornea with ultraviolet light and riboflavin administration as a new treatment for erosive corneal processes, preliminary results in four patients. *Klin Monbl Augenheilkd* 2000;217:190–193.
9. Yaghouti F, Nouri M, Abad JC, et al. Keratoprosthesis: Preoperative prognostic categories. Cornea 2001;20:19–23.
10. Herbert E Kaufman, Bruce A Barron, Marguerite B McDonald, Stephen C Kaufman. The Cornea, Second Edition. Boston, Massachusetts, Butterworth- Heinemann, 1999.
11. Jones BR. Principles in the management of oculomycosis. XXXI Edward Jackson memorial lecture. *Am J Ophthalmol* 1975;79:719-751.
12. Leena Bajracharya, Reeta Gurung. Outcome of therapeutic penetrating keratoplasty in a tertiary eye care center in Nepal. *Clin Ophthalmol* 2015;9 2299–2304.
13. Shi W, Wang T, Xie L, Li S, Gao H, Liu J, Li H. Risk factors, clinical features and outcomes of recurrent fungal keratitis after corneal transplantation. *Ophthalmol* 2010;117(5):890-896.
14. Wollensak G. Crosslinking treatment of progressive keratoconus: new hope. *Curr Opin Ophthalmol* 2006;17:356-360.
15. Spoerl E, Wollensak G, Seiler T. Increased resistance of cross linked cornea against enzymatic digestion. *Curr Eye Res* 2004;29:35-40.
16. Schilde T, Kohlhass M, Spoerl E. Enzymatic evidence of the depth dependence of stiffening on riboflavin/UVA treated cornea. *Ophthalmol* 2008;105:165-169.
17. UC MH, Scott JF. Effects of ultraviolet light on the biological functions of transfer RNA. *Biophys Res Commun* 1966;22:459-465.
18. Kumar V, Lockerbie O, Kell SD. Riboflavin and UV light based pathogen reduction: extent and consequence of DNA damage at molecular level. *Photochem Photobiol* 2004;80:15-21.

How to cite this article: Popat K, Natarajan R. Safety and efficacy of collagen cross linking for donor corneal button in therapeutic penetrating keratoplasty. *Indian J Clin Exp Ophthalmol* 2019;5(1):61-65.