Original article

Collagen cross-linking in recalcitrant corneal ulcers: A case series

Nisha Agrawal, S K Singh
Biratnagar Eye Hospital, Biratnagar, Nepal

Abstract

Introduction: Corneal ulcer is a leading cause of blindness worldwide. Many of these patients don't respond to conventional treatment with topical agents. Collagen cross-linking (CXL) has been suggested to avoid complications requiring emergency keratoplasty. **Subjects and methods:** Six eyes with presumed bacterial keratitis not responding to conventional treatment underwent CXL with ultraviolet A rays and transepithelial riboflavin. Patients with Descematocele and perforated ulcers were excluded. Preoperatively and postoperatively slit lamp examination of cornea and visual acuity recording was done. Postoperative outcome included subjective symptoms like relief in pain, photophobia, lacrimation and objective signs like improvement in epithelisation, corneal scarring with vascularisation. **Results:** Four of the six eyes healed completely with scarring at 2 months follow-up. One of the patients developed Descematocele on 12 days which perforated later. Other patient developed Descematocele on 20 days post CXL. Of the subjective symptoms, pain and epiphora improved in all the patients except one. Photophobia improved only a week after CXL in four out of six patients. Epithelial defect completely healed over time in four out of six cases. All the cases who responded to treatment developed superficial and deep vascularisation of the cornea. Decrease in corneal edema and scarring was noted in four out of the six cases. **Conclusion:** The collagen cross-linking has a beneficial role as an adjuvant to medical therapy in recalcitrant bacterial keratitis. It helps in relief of pain and healing of ulcer. Larger randomized control trails with longer follow-up are required to come to a definite conclusion.

Keywords: cross-linking, recalcitrant ulcer, epithelization

Introduction

Corneal ulcer is one of the most commonly encountered ocular diseases in our country. It is a major cause of corneal blindness worldwide (Whitcher et al, 2001). Many of these patients are successfully treated with topical antimicrobial agents. However, many advanced cases don't respond to this traditional treatment regimen. Recently increasing number of cases have used a combination of riboflavin and ultraviolet-A (UV A) irradiation as adjuvant therapy in refractory keratitis (Price, 2012;Chan et al, 2014; Hafez, 2014).

Collagen cross linking (CXL) technology is called PACK-CXL (Photo-Activated Chromophore for infectious Keratitis cross-linking). Its antimicrobial action is due to photo-oxidative damage induced by reactive oxygen species (Sato K et al, 1995), causing direct cell wall damage of pathogens. Secondly,

Received: 12/10/15 Accepted: 10/12/15
Address for correspondence
Dr Nisha Agrawal, Ophthalmologist.
Biratnagar Eye Hospital, Biratnagar 17, Morang, Nepal
Tel: 9852049870, Fax: 021436359
Email: drnisha_agr@hotmail.com

direct intercalation of the chromophore to the DNA of the pathogen, causing irreversible binding and suppression of replication. It also strengthens the cornea by changing 3 dimensional structures of collagen fibres (Alio JL et al, 2013) and increasing corneal resistance to degradative enzymes (Spoerl E et al, 2004). Another potential advantage of CXL is its toxic effect on inflammatory cells which may limit the inflammatory response to infectious organism (Wang F, 2008).

CXL is possibly a means to promote healing of recalcitrant corneal ulcers. It reduces complications and need for emergency keratoplasty. In circumstances like culture negative cases, non-compliant patients, antimicrobial multiresistance and panresistance, CXL may be more advantageous than conventional therapy (Hafez, 2014).

Herein, we present our initial experience with CXL, as an adjuvant therapy in 6 patients with recalcitrant corneal ulcers not responding to conventional medical management.

Subjects and methods

Seven patients with duration of symptoms of more than 10 days who didn't respond to topical treatment with fortified cefazolin (5%) and gentamicin (1.3%) every half hourly at Biratnagar eye hospital in the month of June 2015 were included.

Baseline visual acuity was recorded. Detailed evaluation of ulcer was done on the slit lamp. Corneal scrapping was submitted for microbiological evaluation i.e. staining with Grams stain and potassium hydroxide (KOH) mount. Bacterial ulcers of moderate severity (infiltrate with an overlying epithelial defect of 3mm in its shortest dimension) or more were included whereas fungal ulcers, Descematocele and perforated ulcers were all excluded. Use of flourescein sodium at the day of surgery was prohibited as it has been proposed to compete with riboflavin for the absorption of UV A irradiation and may thus reduce its antimicrobial effect (Richoz et al, 2013).

OptoXlink 1.0 cross linking device (UV-X A irradiation 365±5nmSD) via 9mm aperture at 45mm distance from apex of cornea was used. Parameters used were T (time): 30 minutes, D (dose): 5.405J/c, P (power): 1.51mW and I (intensity): 3.003mW/cm^2 (McQuaid R et al, 2013).

Surgical procedure

Xylocaine 4% was used topically for anesthesia every 5 minutes for 15 minutes prior to the procedure. Lid speculum was applied. Lights were turned off so that the composition of riboflavin was not affected. Riboflavin sodium phosphate 1 mg (isotonic 0.1% plus dextran, Figure 1) was instilled every 3 minutes for 30 minutes prior to the procedure.

During irradiation riboflavin was continuously instilled at every 3 minutes interval. At the end of the procedure, eye was flushed with normal saline and ciprofloxacin ointment was applied. No contact lens was applied. Finally, the eye was covered with a patch (Figure 2).

Figure 1: Riboflavin used prior to and during cross-linking

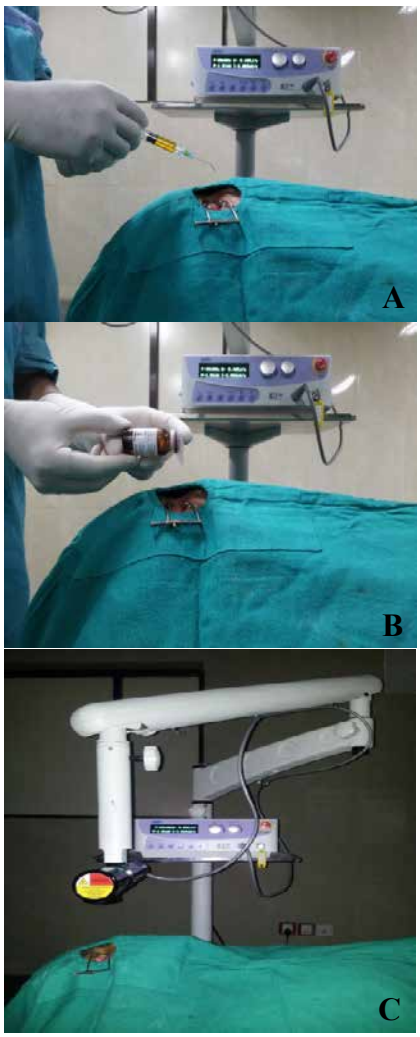


Figure 2: Surgical procedure.
A-riboflavin application.
B- xylocaineapplication.
C-delivery of UV A irradiation

Postoperatively, cycloplegic thrice daily and fortified antibiotics were continued every half hourly similar to the pre-operative period which was gradually tapered when there were signs of improvement.

Initially the patients were followed up daily during the first week, thereafter weekly for 1 month and then 2 weekly for 2 months. Visual acuity and slit lamp examination of ulcer was performed. Subjective improvement in terms of decrease of pain, epihora and photophobia were noted in all patients. Also improvement in epithelisation, ongoing vascularisation and scarring were noted on each follow-up.

Results

Out of seven cases, one patient did not turn up for follow-up and was excluded. Of the six cases included, mean age of patients was 41.33 ± 21.79 years (range: 18-75years). Male to female ratio was 2:1. Mean duration of onset of symptoms at presentation was 25.5±18.35 days (range: 10-60 days). All were moderate to severe grade ulcers. All of the patient's visual acuity at presentation was counting finger close to face (CFCF) to perception of light (PL). All of them had a negative KOH mount. Only one patient showed gram positive cocci on Gram's stain, rest all were negative. Patient data and pre-procedure details of all the patients are given in Table 1.

Table 1: Patient details and ulcer characteristics at the time of presentation

S.no	Age/Gender	Duration (days)	Eye	VA (Day 0)	Dimension (mm X mm)	Gram's	KOH
1	18/M	20	L	HM	6 X 6	-	-
2	45/M	60	L	CFCF	8 X 8	-	-
3	55/M	10	R	HM	5 X 5	GPC	-
4	75/F	21	R	PL	4 X 4	-	-
5	20/F	12	L	PL	8 X 8.5	-	-
6	35/M	30	R	PL	9 X 9	-	-

M- male, F- female, VA- visual acuity, CFCF- counting finger close to face, HM- hand movement, PL- perception of light, L- left, R-right, GPC- Gram positive cocci

All the patients experienced improvement in subjective symptoms. Pain and epiphora improved in all the patients except one, since the very next day. However, photophobia showed gradual improvement only a week after the procedure in four out of the six patients.

Epithelial defect completely healed over time after irradiation with UV- A rays and riboflavin in four out of the six cases. All the cases who responded to treatment developed superficial and deep vascularisation of the cornea. Decrease in corneal edema and scarring was noted in four out of the six cases. (Table 2)

Table 2: Changes in subjective and objective signs following collagen crosslinking

S.no.	Subjective symptoms			Clinical signs		
	Pain	Epiphora	Photophobia	Epithelial defect (mmXmm) (Duration)	Vascularization	Scarring
1.	-	-	-	Healed	+	+
2.	-	+	+	Descematocele (8X4) (1month)	+	-
3.	-	-	-	Healed	+	+
4.	-	-	-	Healed	+	+
5.	-	-	-	Healed	+	+
6.	+	+	+	Perforation (8X8) (1month)	-	-
+ = present, - = absent						

Four of the ulcers healed completely with decrease in pain, ciliary injection, complete re-epithelisation, corneal scarring and vascularisation (Figure 4) after one month. None of these patients had a gain in visual acuity which was because of corneal vascularisation and scarring. One of the remaining two patients developed complete corneal sloughing with large corneal perforation three days after CXL (Figure 5). Lateral tarsorraphy was performed and the eye healed with formation of anterior staphyloma over a period of one month. Remaining one of the cases developed Descematocele two weeks after the procedure (Figure 6). Tissue adhesive and bandage contact lens was applied for tectonic support. Treatment outcome details have been summarized in Table 3.

Table 3. Summary of treatment outcome following collagen cross linking

S.No.	Pre-op VA	Final VA	Outcome
1	HM	HM	Healed
2	CFCF	PL	Desmatocele
3	HM	HM	Healed
4	PL	HM	Healed
5	PL	HM	Healed
6	PL	PL	Perforation
VA- visual acuity, CFCF- counting finger close to face, HM- hand movement, PL- perception of light			

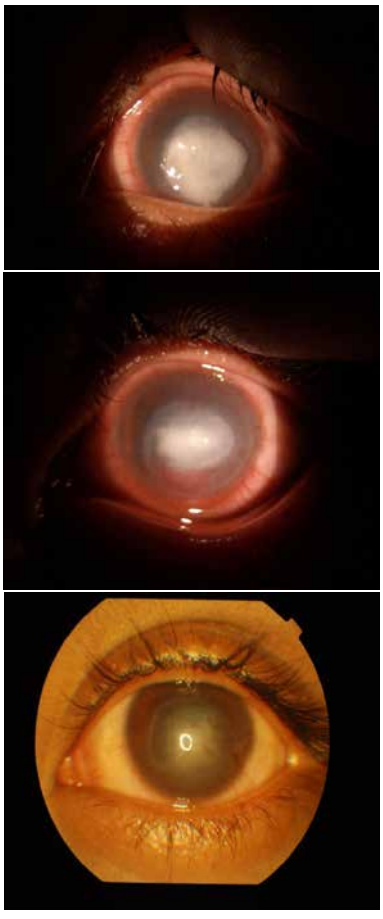


Figure 4: A case of healed keratitis. 4A is preoperative photo. 4B at 20 days postoperative followup. 4C is at 2 months follow-up with complete healing of infiltration with vascularisation and scar formation.

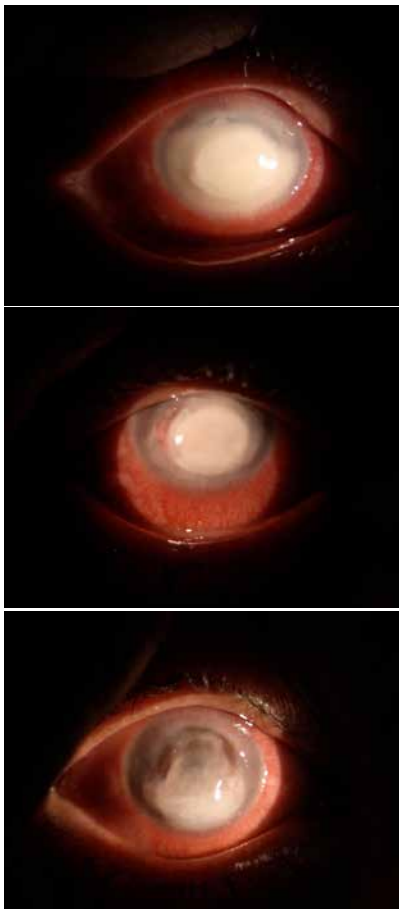
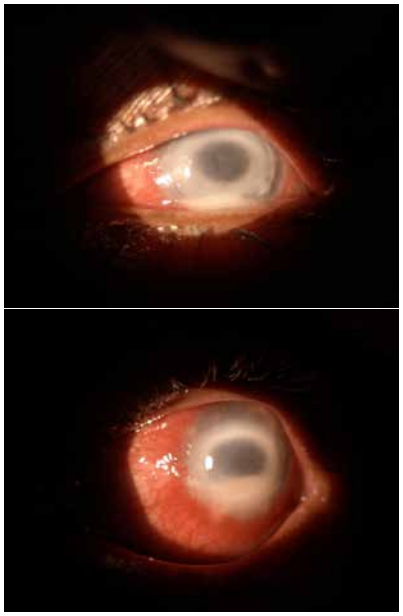


Figure 5: A case of perforated keratitis. 5A is preoperative photo. 5B is at 12days postoperative follow-up with Descematocele formation. 5B is at 1month follow-up with total sloughing and perforation.



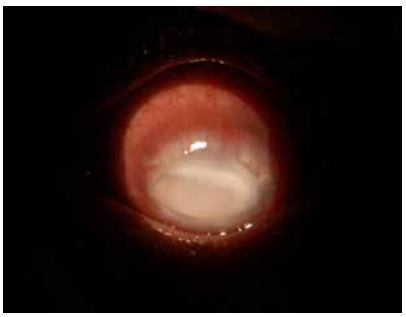


Figure 6: Case with formation of Descematocele. 6A Preoperative photo. 6B at 20days follow-up. 6C at one month follow-up with Descematocele formation

Discussion

The role of collagen cross linking in corneal ulcer is still struggling hard to find its identity in this growing competitive market. Many reports have supported its use in bacterial keratitis, with more variable outcomes in cases of fungal, acanthamoeba and viral keratitis (Ferrari et al, 2013).

Treatment success was noted in 4 of our patients (66.67%). This case series has proved that CXL aids in healing of recalcitrant bacterial corneal ulcers. Similar success stories of CXL treatment in recalcitrant keratitis have been given by various authors. Anwar et al (2011) also reported that cross-linking is useful for management of non-healing corneal ulcer. Another study by Iseli et al (2008) reported five cases of corneal ulcer in which progression of corneal melting was interrupted in all the cases (100%) due to CXL and hence preventing the need for emergency keratoplasty.

Chan et al (2014) also concluded beneficial role of collagen cross linking in recalcitrant corneal ulcers in their case series of 4 cases with two culture proven bacterial corneal ulcers, one unidentified organism and one Acanthamoeba keratitis. But, all of our patients were presumed bacterial keratitis and other etiologic factors were not included. In his case series, more than one session of CXL was performed in all the cases in comparision to our study in which only a single session of CXL was performed.

A meta-analysis of 12 articles was also conducted by Alio et al (2013) in which they reviewed 104 cases of corneal ulcer treated with CXL. The analysis proved that the suggested therapy had a favorable result in 85% of cases and 15% (16 cases) required keratoplasty. Similarly, in our study also the therapy proved to be effective in 66.6% of cases.

In corneal ulcer due to other etiological factors, a prospective study was conducted by Price et al (2012) regarding CXL in 40 cases of infectious keratitis. They concluded that the success rate was higher for cases of bacterial keratitis than for fungal and Acanthamoeba keratits. They also reported its correlation with depth (less than 250μm) and size of infiltrate (less than 2.5mm in diameter). Comparing this finding with our cases, all of our patients had a larger infiltrate. The exact depth of ulcer could not be ascertained due to unavailability of anterior segment optical coherence tomography.

Zamani et al (2015) conducted a study in 8 patients of which 3 were Pseudomonas infection. Six out of 8 and all the Pseudomonas cases responded to this treatment regimen. Hence, efficacy and treatment success was proved in Pseudomonas also.

CXL in fungal keratitis has questionable role. Uddaraju M et al (2015) very recently performed a randomized control trial of adjuvant use of CXL in Fusariumkeratits. The treatment group showed more corneal perforation than control group concluding CXL failure in deep stromal keratitis.

Two of the cases did not respond to CXL which may be due to late presentation of the cases (30 days and 60 days), very large ulcers (8 X 8 mm^2 and 9 X 9 mm^2) and deep involvement of the cornea. Various authors at various time have also proposed that the stiffening effect of crosslinking is limited to <300μm of the cornea (Kohlhaas et al, 2008; Panda et al, 2012; Price et al, 2012). This could also be the cause of treatment failure in two cases.

There was improvement in clinical symptoms and signs in our cases. Hafez et al (2014) studied these symptoms and signs in their series of CXL in 5 cases. They also reported similar findings regarding relief of pain, epiphora and improvement in re-epithelisation in all of their cases. But in this study ulcers with corneal thickness less than 400 μm were excluded. However, in our study corneal pachymetry could not be performed due to unavailability of non-contact pachymetry method and deep ulcers were also included.

Another topic of controversy is whether CXL should be used primarily or as an adjuvant therapy. A comparison was made in between CXL with conventional medical treatment and conventional medical treatment alone by Said et al (2014). Time for resolution was similar in both the groups. One recurrence and three perforations were noted in the control group. So, the authors suggested adjuvant role of CXL in reducing the risk for complications from severe infectious keratitis.

Comparison could not be made with cases treated in this geographic distribution as no similar case reports are available from Nepal.

Conclusion

The collagen cross-linking has a beneficial role as an adjuvant therapy in addition to medical therapy in recalcitrant bacterial keratitis. It improves clinical symptoms and signs and helps in healing of ulcer with scar formation. Larger randomized control trials with longer follow-up are required to come to a definite conclusion.

References

Alio JL, Valle DD, Castillo JMB, Fenandez JAG (2013). Corneal collagen cross linking and infectious keratitis; A systematic review and meta-analysis of reported cases. Journal of Ophthalmic Inflammation and Infection; 3:47.

Anwar H, El–Danasoury A, Hashema (2011). Corneal collagen crosslinking

in the treatment of infectious keratitis. ClinOphthalmol; 5:1277-1280.

Chan E, Snibson GR, Sullivan L (2014). Treating of infectious Keratitis with riboflavin and ultraviolet A irradiation. J Cataract Refract Surg; 40:1919-1925.

Ferrare G, Luliano L, Vigano M, Rama P (2013). Impending corneal perforation after collagen crosslinking for herpetic keratitis. J Cataract Refract Surg;39:636-641.

Hafez M (2014). Evaluation of the therapeutic effect of corneal collagen cross-linking in the treatment of resistant corneal ulcer. J Egypt OphthalmolSoc; 107: 187-190.

Iseli HP, Thiek MA, Hafezi F et al (2008). Ultraviolet A/ riboflavin corneal crosslinking for infectious keratitis associated with corneal melts. Cornea; 27:590-594.

Kohlhaas M, Spoerl E, Schilde T et al (2006). Biochemical evidence of the distribution of cross-links in corneas treated with riboflavin and ultraviolet A light. J Cataract and refract Surg; 32(2):279-283.

Panda A, Krishna SN, Kumar S (2012). Photo-activated riboflavin therapy of refractory corneal ulcers. Cornea;31:1210-1213.

Price MO, Tenman LR, Schrier A, Fairchild KM, Trokel SL, Price FW Jr (2012). Photoactivated riboflavin treatment of infectious keratitis using collagen cross-linking technology. J Refract Surg;28:706-713.

McQuaid R,Cummings AB, Mrochen M (2013).Thetheoryandartofcornealcrosslinking. Indian J Ophthalmol;61(8):416-419.

Richoz O, Gatzioufas Z, Francois P, Schrsnzel J, Hafezi F (2013). Impact of flourescein on the antimicrobial efficacy of photoactivated riboflavin in corneal collagen cross-linking. J Refract Surg; 29:842-845.

Said DG, Elalfy MS, Gatzioufas Z, Al-Zakzouk ES, Hassan MA, Saif MY et al (2014). Collagen cros-slinking with photoactivated riboflavin (PACK-CXL) for the treatment of advanced infectious keratitis with corneal melting. Ophthalomlogy;121:1377-1382.

Sato K, Taguchi H, Maeda T, Minami H, Asada Y, Watanabe Y et al (1995). The primary cytotoxicity in ultraviolet-A irradiated riboflavin solution is derived from hydrogen peroxide. J Invest Dermatol;105:608-612.

Spoerl E, Wollensak G, Seifer T (2004). Increased resistance of cross-linked cornea against enzymatic digestion. Curr Eye Res;29:35-40.

Uddaraju M, Mascarenhas J, Das MR, Radhakrishnan N, Keenan JD, Prajna L et al (2015). Collagen Cross-linking as an adjuvant therapy in the management of recalcitrant deep stromal fungal keratitis: A randomized trial. Am J Ophtahlmol;160(1):131-134.

Wang F (2008). UV A/ riboflavin-induced apoptosis in mouse cornea. Ophthalmologica;222:369-372.

Whitcher JP, Srinivasan M, Upadhyay MP. Corneal blindness: a global perspective. Bull World Health Org 2001; 79:214–221.

Zamani M, Bazaz MP, Assadi M (2015). Corneal collagen crosslinking for treatment of non healing corneal ulcers.J Ophthalmic Vis Res; 10 (1): 16-20.

Source of support: nil. Conflict of interest: none