Collagen Cross-linking for Microbial Keratitis

Prashant Garg, Sujata Das¹, Aravind Roy²

Abstract:
Collagen cross-linking is gaining popularity not only for arresting the progression of keratoconus but also other indications including management of corneal infections. In this review article, we analyzed the published literature to understand the level of evidence for its use in corneal ulcer. Photoactivated riboflavin and ultraviolet A light are known to possess antimicrobial properties. The treatment also induces formation of inter- and intra-fibrillar bonds, thereby making the corneal collagen resistant to the action of proteases arresting stromal melt. Both properties are well documented in in vitro experiments. The antimicrobial action is seen against bacteria, fungi, and parasites. The animal experiments have documented its efficacy against bacterial and fungal keratitis models. The literature on its application in human corneal infection is highly variable and comprises case reports, case series, and comparative nonrandomized and randomized trials. The treatment has been used as primary treatment, adjunctive treatment along with antibiotics, as the first line of treatment as well as for failed medical treatment cases. Even the cases included are of variable severity caused by a variety of microorganisms including culture-negative cases. Furthermore, the treatment protocols are also variable. While most reports show beneficial effects for bacterial corneal ulcer cases, especially those with superficial infiltrate, the effect has been mixed for fungal and parasitic keratitis. In view of these characteristics, we infer that the level of evidence for its use in corneal ulcer is at most weak. We need well-characterized, high-quality, clinical trials of sufficient power to assess its true value.

Keywords:
Acanthamoeba, bacteria, collagen cross-linking, fungi, microbial keratitis, riboflavin, stromal melt

Introduction
Corneal collagen cross-linking (CXL) is a procedure, wherein a photosensitizer (riboflavin) and ultraviolet (UV) light are used to strengthen cornea by promoting formation of inter- and intra-fibrillar covalent bonds. The currently employed CXL techniques were developed by researchers at the University of Dresden in the late 1990s.¹ The experiments showed that cross-linked porcine and rabbit corneas were stiffer and more resistant to enzymatic digestion. The corneas were also shown to contain higher molecular weight polymers of collagen due to fibril cross-linking. Safety studies showed that the procedure is safe if the corneal thickness exceeded 400 µ.

In 2003, human studies began in Dresden, and early results were promising. Since then, the procedure is gaining popularity among physicians across the globe. It was initially developed for halting progression of keratoconus. But over time, indications of its use kept on expanding and physicians started exploring the possibility of its use in corneal infections. However, while there is a general agreement on safety and efficacy of its use in ectatic disorders of the cornea and the treatment protocols standardized, the use for infectious keratitis has attracted a great amount of debate. Therefore, it will be prudent to review published literature and generate evidences in favor or against its use for corneal infection.

The aims of this review article are to:
• Understand the basis of its use in corneal infections

How to cite this article: Garg P, Das S, Roy A. Collagen cross-linking for microbial keratitis. Middle East Afr J Ophthalmol 2017;24:18-23.
The authors found that the growth inhibition was primarily due to 
combined riboflavin and UVA exposure compared to UVA alone. However, the inhibition effect was not 
noticed for Candida albicans. The authors concluded that 
CXL using riboflavin and UVA light can be new potential 
treatment for infectious keratitis.

Several other authors have also carried out similar in vitro 
experiments. While most studies demonstrated positive 
results, Kashiwabuchi et al. found no activity against 
oxacillin-susceptible Staphylococcus aureus.[5-9]

Richoz et al. conducted a study to analyze whether optimized 
photoactivated chromophore treatment maintains 
antibacterial efficacy.[8] The results of the study showed 
that the antibacterial efficacy follows the Bunsen-Roscoe 
law of reciprocity and can be maintained even when the 
irradiation intensity is considerably increased.

In yet another experiment, Bäckman et al. found that 
longer UVA exposure time in combination with lower 
riboflavin levels was more efficient in killing bacteria 
compared to the standard cross-linking settings.[10]

Riboflavin/UVA treatment has been found to be effective 
against fungal pathogens as well. Experiments conducted 
by Sauer et al. on the following three groups of fungi: C. 
albicans, Fusarium sp., and Aspergillus fumigatus showed 
that these strains did not show any increased GIZ 
without previous amphotericin-B medication. However, 
GIZ was significantly greater after pretreatment with 
amphotericin-B and exposure to riboflavin/UVA. The 
authors concluded that CXL enhances the effect of 
amphotericin-B on fungal cell membranes.[11]

Qualitative and quantitative measurements of C. albicans 
and Fusarium solani strains after exposure to CXL 
treatment showed a significant decrease in numbers and 
alterations of cell morphology.[12]

Del Buey et al. evaluated antimicrobic activity of CXL 
and found that single application of the treatment does 
not eradicate Acanthamoeba.[13] Makdoumi et al. also 
found that the growth inhibition was primarily due to 
antiprotazoal effect of UVA light, and riboflavin does 
not amplify it further.[14]

Antimicrobial properties
In the 1960s, Tsugita et al. reported that the application of 
riboflavin and UVA light leads to inactivation of tobacco 
mosaic virus.[3] Since then, this knowledge has been used 
to eradicate microorganisms such as in sterilization of 
water, surfaces, food, and blood product. The release of 
reactive oxygen species on activation of riboflavin by 
UVA light results in damage to DNA and RNA 
of pathogens. In addition, UV irradiation on its own 
damages nucleic acid and has virucidal, bactericidal, 
and fungicidal effects.

Martins et al. studied antimicrobial properties of 
riboflavin and UVA light against a variety of keratitis 
pathogens including antibiotic sensitive and resistant 
or ganisms.[14] The authors found that the growth 
inhitation zone (GIZ) was significantly greater with 
combined riboflavin and UVA exposure compared 
to UVA alone. However, the inhibition effect was not 
noticed for Candida albicans. The authors concluded that...
a microcomputer-controlled biomaterial tester showed that the procedure results in increase in mechanical rigidity in porcine and human corneas. The increase was greater in human corneas. In another study, the same group postulated that the increased biomechanical rigidity is secondary to increase in diameter of corneal collagen. He et al. who used quantitative noninvasive ultrasound method reproduced similar findings. Spoerl et al. used ocular response analyzer software and found that keratoconic corneas show altered biomechanical properties after cross-linking. Spoerl et al. also showed that porcine corneas after CXL showed a marked increase in resistance to enzymatic digestion when exposed to pepsin, trypsin, and collagenase solutions compared to controls.

In addition to these properties, studies have also found riboflavin to have anti-inflammatory and nociceptive effects. The later was corroborated by Shetty et al. who reported a significant decrease in pain following CXL in patients of refractory advanced microbial keratitis.

Overall, the laboratory data suggest that CXL of the cornea using riboflavin and UVA light not only has potential for eliminating offending pathogens but also confers corneal rigidity that reduces its susceptibility to proteolytic enzymes and corneal melt. In addition, it has anti-inflammatory effect. All of these effects justify its use in the management of corneal ulcer.

Are these Results Translated in In vivo Animal Experiments?

Review of literature in English language using PubMed showed that few studies have been carried out to evaluate the efficacy of CXL in animal corneal infection models.

Tal et al. evaluated the efficacy of corneal CXL as primary therapy for S. aureus corneal ulcer in rabbit model. Forty rabbits were randomly assigned to four groups: group-A: No treatment (control); Group-B: Topical antibiotic treatment (cefazolin 50 mg/mL, Garamycin 14 mg/mL drops, and chloramphenicol 5% ointment every 2 h); Group-C: CXL; and Group-D: CXL + topical antibiotics. After 1 month of treatment, Group-C ulcers had the smallest mean scar diameter and shortest mean healing time.

Cosar et al. studied the effects of CXL on the penetration of topical 0.5% moxifloxacin, number of colony-forming units (CFUs), and the clinical course of infection in Pseudomonas aeruginosa rabbit keratitis model. Although there was a significant difference in the clinical improvement between moxifloxacin group and moxifloxacin with CXL group, there was no difference in CFU counts between two groups. Authors concluded that the synergistic effect of CXL as reflected by clinical cure in the treatment of corneal ulcer is not just through enhanced antibiotic penetrance or efficacy.

CXL treatment has also been studied in rabbit models of Fusarium. After the infection was established, the rabbits were divided into two groups - one group did not receive any treatment (control) while another group underwent CXL. The CXL group showed superior clinical improvement and has significantly less fungal load as demonstrated by the number of CFUs compared to controls. In addition, evaluation of excised corneas showed that CXL-treated corneas had fewer Fusarium hyphae and inflammatory cells.

CXL treatment, however, was not found effective in rabbit model of Acanthamoeba keratitis.

What is the Evidence of Its Use in Human Corneal Infection?

The use of CXL for keratoconus established the safety and efficacy of the procedure in strengthening corneal collagen. This along with the theoretical basis mentioned above prompted clinicians to use CXL for microbial keratitis cases as well. This resulted in a variety of publications ranging from case reports to large and small case series and even prospective randomized or nonrandomized clinical trials. The authors used CXL as sole primary treatment, adjuvant to specific medical treatment, and as supplemental treatment in failed medical treatment cases. The procedure has been tried against a variety of pathogens including drug sensitive and resistant bacteria, fungi, and parasites. Few authors have even attempted systematic review or meta-analysis of the published literature. A wide variety in the publications clearly points that the application of CXL for this indication is still evolving and our understanding of its level of effectiveness still not mature.

Since Theo Seiler group took lead and carried out laboratory experiments to understand fundamental basis of the use of CXL for corneal infection, they were the first to publish its use in human corneal infection. They reported CXL treatment in five patients of infectious keratitis that did not respond to systemic and topical antibiotic therapy and documented that the progression of corneal melting was halted and the infiltrate size reduced in four of five patients after CXL treatment. The authors concluded that the procedure may be used in therapy-resistant cases to avoid emergency keratoplasty.

Similar results on using CXL as adjunctive treatment in medically resistant cases have been published by other authors. Shetty et al. have used CXL treatment in 15 eyes of microbial keratitis caused by bacteria and fungi. The patients received treatment...
with antibiotics/antifungals for 2 weeks before CXL treatment. Six of nine patients with bacterial keratitis and three of six patients with fungal keratitis resolved following CXL treatment. Patients with deep stromal keratitis or endothelial plaque failed to resolve. All patients had resolution of pain on the 1st postoperative day. There was an appearance of or increase in hypopyon in seven patients. The authors concluded that CXL can be used as adjunctive therapy in nonresolving microbial keratitis with superficial stromal involvement.

Panda et al. published its use in seven refractory corneal ulcers managed medically for 4–12 weeks.[29] In all cases, the progression of corneal melting was halted after CXL treatment. Skaat et al. reported six patients with severe refractory infectious keratitis treated with CXL.[30] In five cases, signs of infection and inflammation resolved within 1–2 weeks after the treatment. However, one patient required penetrating keratoplasty. Sorkhabi et al. used CXL therapy for resistant corneal ulcers and concluded that CXL is a viable therapeutic option for the treatment of corneal ulcers and can be used as an adjuvant in resistant cases.[31]

Can Corneal Collagen Cross-linking Be Used in Combination with Antibiotics as Primary Treatment?

Price et al. published a prospective case series of forty patients with microbial keratitis treated by CXL while continuing on standard antimicrobial treatment.[32] The primary outcome measures were the time to resolution of the infiltrate and the epithelial defect. In six patients, the keratitis did not resolve, and the eyes required penetrating keratoplasty. The authors concluded that the CXL is most effective in superficial infections and bacterial etiology. The authors also cautioned that it should be avoided in viral infections.

Can it Be Used as Sole Treatment without Antibiotics?

Makdoumi et al. have described the use of CXL as a primary therapy for bacterial keratitis.[33] In a prospective nonrandomized study, they included 16 patients of microbial keratitis without primary treatment. All eyes responded to the photochemical treatment with improvement in symptoms and signs. Epithelial healing was achieved in all cases. Antibiotic administration was necessary in two cases only. In addition, one patient required amniotic membrane transplant. One thing to note, however, is that the size of infiltrate in this series was much smaller.

What is the Experience with Fungal and Parasitic Infections?

In addition to the cases included in case series mentioned in the previous sections, Li et al. reported successful use of CXL for eight cases of microbiologically proven fungal keratitis.[34] CXL treatment was performed between 5 days and 1 month from the time of initial presentation. The healing of corneal epithelium and ulcer was achieved in all cases 3–8 days after CXL treatment. None of the cases required corneal transplantation.

On the contrary, Uddaraju et al. in a prospective randomized clinical trial found that CXL is not helpful in deep stromal fungal keratitis.[35] Five eyes in the CXL group and three eyes in the non-CXL group experienced treatment failure by 6 weeks (P = 0.56). In a secondary analysis, the CXL group experienced more perforations than the non-CXL group (4 vs. 0, respectively; P = 0.02). Vajpayee et al. in a retrospective analysis also found no difference in average healing time and final visual acuity between groups with medical management alone and medical management with CXL.[36] The authors concluded that CXL as an additional treatment has no advantage over medical treatment alone for moderate fungal keratitis cases.

Experience with CXL for *Acanthamoeba* keratitis is very limited. In a recently published meta-analysis, Papaioannou et al. found that 10 of 11 eyes were successfully managed using CXL as adjunctive treatment.[37] Most of these cases were reported as a case report or small series, and therefore, the evidence is insufficient to draw valid conclusions.[38,39] Is There Any Randomized Comparative Clinical Trial Assessing the Efficacy of the Procedure?

As of 2015, two prospective comparative clinical trials assessing the efficacy of CXL compared to conventional treatment have been published. Said et al. included forty patients with advanced infectious keratitis coexisting with corneal melting due to bacteria, fungi, *Acanthamoeba*, and mixed organism.[28] Twenty-one patients underwent CXL treatment in addition to antimicrobial therapy. The control group consisted of 19 patients who received antimicrobial therapy only. CXL with photoactivated riboflavin did not shorten the time to corneal healing. However, the complication rate of perforation was more in the control group while none of the cases in CXL group developed the complication. The authors concluded that CXL is an effective adjuvant therapy in the management of severe infectious keratitis associated with corneal melting and prevents corneal perforation.

In another randomized comparative clinical trial, Bamdad et al. randomized 32 moderately severe corneal ulcer cases into those who received CXL in addition to medical treatment and controls who were treated using standard medical treatment alone.[40] The mean treatment
duration as well as epithelial defect and infiltrate size on day 7 and 14 was smaller in cases compared to controls. The authors concluded that CXL has a beneficial effect in patients with moderate bacterial keratitis and accelerates resolution of infiltrate, promotes epithelial healing, and shortens the course of treatment.

Are There Other Issues that Must Be Taken into Consideration While Evaluating the Level of Evidence in Favor or against the Use of Corneal Collagen Cross-linking for Microbial Keratitis?

After going through the preceding section, one will be inclined to infer that there is sufficient evidence in favor of the use of CXL for microbial keratitis. It not only prevents stromal melt and corneal perforation but also promotes resolution of ulcer including those cases that otherwise fail to respond to medical treatment.

However, we need to exercise caution in making these conclusions. The reasons are:

• The evidence is weak. As of 2016, only two prospective comparative studies evaluating efficacy of CXL in human corneal ulcer have been published. Of these, only one used randomization. The sample size was inadequate in both of these. Therefore, most of the evidence is in the form of case reports and case series that is considered weak evidence.

• The reports describe cases with variable disease severity from very small superficial infiltrate to moderately severe keratitis with stromal melt. No standard or well-defined definitions of severity were used in these reports.

• The authors have included keratitis cases caused by a wide variety of organisms including bacteria, fungi, parasites, herpes simplex virus, and culture-negative cases. While the number of bacterial keratitis cases is high enough, the literature provides only limited experience for infection by other organisms.

• Authors in these studies have used variable protocol of CXL treatment. While majority have used conventional protocol with irradiation of 365 nm and 3 mW/cm² duration of exposure was variable. There is a report of using accelerated protocol (365 nm and 9 mW/cm² for 10 min) in a case of fungal keratitis.

• The definition of treatment failure for subjecting a case to CXL treatment was not uniform. This is obvious from the fact that the duration of treatment before enrollment in the CXL studies ranged between 1 and 168 days in bacterial ulcer, 1 and 84 days for fungal, and 1 day and 1 year for Acanthamoeba keratitis cases.

• The term “stromal melt” is vague, and the data on residual stromal thickness or extent of melt are not provided. Therefore, one cannot make conclusions on its safe use in the presence of severe stromal loss and thinning. The experience in keratoconus suggests that the procedure is safe only if the corneal thickness is at least 400 µ. Does that apply to microbial keratitis as well cannot be answered based on the published literature. Further, the stromal loss in corneal infection is not uniform, and the possibility of finding areas of significant stromal loss and normal thickness within the same cornea cannot be ruled out.

• The benefits of CXL treatment for fungal and Acanthamoeba are not uniform. Laboratory, animal, and human experience is mixed and does not provide a clear evidence in favor or against its use for these cases.

Conclusions

The collagen cross-linking seems promising and can provide an alternative adjunctive treatment option in the management of corneal ulcer, especially those caused by bacteria and associated with superficial infiltrate. The use of CXL in fungal and Acanthamoeba keratitis seems to be controversial and best avoided. We need more research, especially using appropriately powered randomized controlled trials to assess its true value. It will also be important to use well-characterized protocols including case definition, inclusion and exclusion criteria, and outcome measures. Further, it will also be important to publish both positive and negative results.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1. Ashwin PT, McDonnell PJ. Collagen cross-linkage: A comprehensive review and directions for future research. Br J Ophthalmol 2010;94:965-70.
2. Srinivasan M, Mascarenhas J, Rajaraman R, Ravindran M, Lalitha P, Glidden DV, et al. Corticosteroids for bacterial keratitis: The Steroids for Corneal Ulcers Trial (SCUT). Arch Ophthalmol 2012;130:143-50.
3. Tsugita A, Okada Y, Uehara K. Photosensitized inactivation of ribonucleic acids in the presence of riboflavin. Biochim Biophys Acta 1965;103:360-3.
4. Martins SA, Combs JC, Noguera G, Camacho W, Wittmann P, Walther R, et al. Antimicrobial efficacy of riboflavin/UVA combination (365 nm) in vitro for bacterial and fungal isolates: A potential new treatment for infectious keratitis. Invest Ophthalmol Vis Sci 2008;49:3402-8.
5. Schrier A, Greetel G, Attia H, Trokel S, Smith EF. In vitro antimicrobial efficacy of riboflavin and ultraviolet light on Staphylococcus aureus, methicillin-resistant Staphylococcus aureus, and Pseudomonas aeruginosa. J Refract Surg 2009;25:5799-802.
6. Mkdoumi K, Bäckman A, Mortensen J, Crafoord S. Evaluation of antibacterial efficacy of photo-activated riboflavin using ultraviolet light (UVA). Graefes Arch Clin Exp Ophthalmol 2010;248:207-12.
7. Makdoumi K, Bäckman A. Photodynamic UVA-riboflavin bacterial elimination in antibiotic-resistant bacteria. Clin Exp Ophthalmol 2016;44:582-6.

8. Richoz O, Kling S, Hoogewoud F, Hammer A, Tabibian D, Francois P, et al. Antibacterial efficacy of accelerated photoactivated chromophore for keratitis-corneal collagen cross-linking (PACK-CXL). J Refract Surg 2014;30:850-4.

9. Kashiwabuchi RT, Khan Y, Carvalho FR, Hirai F, Campos MS, McDonnell PJ. Antimicrobial susceptibility of photodynamic therapy (UVA/riboflavin) against Staphylococcus aureus. Arq Bras Oftalmol 2012;75:423-6.

10. Bäckman A, Makdoumi K, Mortensen J, Crafoord S. The efficiency of cross-linking methods in eradication of bacteria is influenced by the riboflavin concentration and the irradiation time of ultraviolet light. Acta Ophthalmol 2014;92:656-61.

11. Sauer A, Lethsch-Bru V, Speeg-Schatz C, Touboul D, Colin J, Candolfi E, et al. In vitro efficacy of antifungal treatment using riboflavin/UV-A (365 nm) combination and amphotericin B. Invest Ophthalmol Vis Sci 2010;51:3950-3.

12. Kashiwabuchi RT, Carvalho FR, Khan YA, Hirai F, Campos MS, McDonnell PJ. Assessment of fungal viability after long-wave ultraviolet light irradiation combined with riboflavin administration. Graefes Arch Clin Exp Ophthalmol 2013;251:521-7.

13. del Buey MA, Cristóbal JA, Casas P, Goñi P, Clavel A, Mínguez E, et al. Evaluation of in vitro efficacy of combined riboflavin and ultraviolet a for Acanthamoeba isolates. Am J Ophthalmol 2012;153:399-404.

14. Makdoumi K, Bäckman A, Mortensen J, Magnuson A, Crafoord S. Comparison of UVA- and UVA/riboflavin-induced growth inhibition of Acanthamoeba castellanii. Graefes Arch Clin Exp Ophthalmol 2013;251:109-14.

15. Wollensak G, Spoerl E, Seiler T. Stress-strain measurements of human and porcine corneas after riboflavin-ultraviolet-A-induced cross-linking. J Cataract Refract Surg 2003;29:1780-5.

16. Wollensak G, Wilsch M, Spoerl E, Seiler T. Collagen fiber diameter in the rabbit cornea after collagen crosslinking by riboflavin/UV-A. Cornea 2004;23:503-7.

17. He X, Spoerl E, Tang J, Liu J. Measurement of corneal changes after collagen crosslinking using a noninvasive ultrasound system. J Cataract Refract Surg 2010;36:1207-12.

18. Spoerl E, Terai N, Scholz F, Raikrup F, Pillunat LE. Detection of biomechanical changes after corneal cross-linking using ocular response analyzer software. J Refract Surg 2011;27:452-7.

19. Spoerl E, Wollensak G, Seiler T. Increased resistance of crosslinked cornea against enzymatic digestion. Curr Eye Res 2004;29:35-40.

20. Kymionis GD, Mikropoulos DG, Fortaliou DM, Voudouragkaki IC, Kozobolis VP, Konstas AG. An overview of corneal collagen cross-linking (CXL). Adv Ther 2013;30:859-69.

21. Shetty R, Nagaraja H, Jayadev C, Shivanna Y, Kugar T. Collagen crosslinking in the management of advanced non-resolving microbial keratitis. Br J Ophthalmol 2014;98:1033-5.

22. Tal K, Gal-Or O, Pillar S, Zahavi A, Rock O, Bahar I. Efficacy of primary collagen cross-linking with photoactivated chromophore (PACK-CXL) for the treatment of Staphylococcus aureus-induced corneal ulcers. Cornea 2015;34:1281-6.

23. Cosar CB, Kucuk M, Celik E, Gonen T, Akyar I, Serteser M, et al. Microbiologic, pharmacokinetic, and clinical effects of corneal collagen cross-linking on experimentally induced pseudomonas keratitis in rabbits. Cornea 2015;34:1276-80.

24. Galperin G, Berra M, Tau J, Boscaro G, Zarate J, Berra A. Treatment of fungal keratitis from Fusarium infection by corneal cross-linking. Cornea 2012;31:176-80.

25. Berra M, Galperin G, Boscaro G, Zarate J, Tau J, Chiariadia P, et al. Treatment of Acanthamoeba keratitis by corneal cross-linking. Cornea 2013;32:174-8.

26. Iseli HP, Thiel MA, Hafezi F, Kampmeier J, Seiler T. Ultraviolet A/riboflavin corneal cross-linking for infectious keratitis associated with corneal melts. Cornea 2008;27:590-4.

27. Makdoumi K, Mortensen J, Crafoord S. Infectious keratitis treated with corneal crosslinking. Cornea 2010;29:1353-8.

28. Said DG, Elalhy MS, Gatziozas Z, El-Zakzouk ES, Hassan MA, Saif MY, et al. Collagen cross-linking with photoactivated riboflavin (PACK-CXL) for the treatment of advanced infectious keratitis with corneal melting. Ophthalmology 2014;121:1377-82.

29. Panda A, Krishna SN, Kumar S. Photo-activated riboflavin therapy of refractory corneal ulcers. Cornea 2012;31:1210-3.

30. Skaat A, Zadok D, Goldich Y, Varsano D, Berger Y, Ezra-Nimni O, et al. Riboflavin/UVA photochemical therapy for severe infectious keratitis. Eur J Ophthalmol 2014;24:21-8.

31. Sorkhabi R, Sedgipoor M, Mahdavifard A. Collagen cross-linking for resistant corneal ulcer. Int Ophthalmol 2013;33:61-6.

32. Price MO, Tenkman LR, Schrier A, Fairchild KM, Trokel SL, Price FW Jr. Photoactivated riboflavin treatment of infectious keratitis using collagen cross-linking technology. J Refract Surg 2012;28:706-13.

33. Makdoumi K, Mortensen J, Sorkhabi O, Malivall BE, Crafoord S. UVA-riboflavin photochemical therapy of bacterial keratitis: A pilot study. Graefes Arch Clin Exp Ophthalmol 2012;250:95-102.

34. Li Z, Jhanji V, Tao X, Yu H, Chen W, Mu G. Riboflavin/ultraviolet light-mediated crosslinking for fungal keratitis. Br J Ophthalmol 2013;97:669-71.

35. Uddaraju M, Mascarenhas J, Das MR, Radhakrishnan N, Keenan JD, Pranja L, et al. Corneal Cross-linking as an adjuvant therapy in the management of recalcitrant deep stromal fungal keratitis: A randomized trial. Am J Ophthalmol 2015;160:131-4.e5.

36. Vajpayee RB, Shafi SN, Maharana PK, Sharma N, Jhanji V. Evaluation of corneal collagen cross-linking as an additional therapy in mycotic keratitis. Clin Exp Ophthalmol 2015;43:103-7.

37. Papaoannou L, Miligos M, Papanastasiou M. Corneal collagen cross-linking for infectious keratitis: A systematic review and meta-analysis. Cornea 2016;35:62-71.

38. Khan YA, Kashiwabuchi RT, Martins SA, Castro-Combs JM, Kalyani S, Stanley P, et al. Riboflavin and ultraviolet light therapy for resistant corneal ulcer. Int Ophthalmol 2013;33:61-6.

39. Khan YA, Kashiwabuchi RT, Martins SA, Castro-Combs JM. Fungal keratitis by corneal cross-linking. Ophthalmology 2011;118:324-31.

40. Bamdad S, Malekhosseini H, Khoosravi A. Ultraviolet A/riboflavin collagen cross-linking for treatment of moderate bacterial corneal ulcers. Cornea 2015;34:402-6.