Contact Lens & Anterior Eye 37 (2014) 224–227

Contents lists available at ScienceDirect

Contact Lens & Anterior Eye

journal homepage: www.elsevier.com/locate/clae

Case report

Corneal cross-linking for *Acanthamoeba* keratitis in an orthokeratology patient after swimming in contaminated water☆

Ángeles Arance-Gil[a], Ángel Ramón Gutiérrez-Ortega[a], César Villa-Collar[b], Amelia Nieto-Bona[c], Daniela Lopes-Ferreira[d], José Manuel González-Méijome[d,*]

[a] *Clínica Oftalmológica Novovision, Paseo de la Castellana, Madrid, Spain*
[b] *Universidad Europea de Madrid, Spain*
[c] *Universidad Complutense de Madrid, Spain*
[d] *Clinical & Experimental Optometry Research Lab (CEORLab), Center of Physics, University of Minho, Braga, Portugal*

ARTICLE INFO

Article history:
Received 28 June 2013
Received in revised form 29 October 2013
Accepted 20 November 2013

Keywords:
Acanthamoeba keratitis
CXL
Orthokeratology
Confocal microscopy
Amniotic membrane
Penetrating keratoplasty

ABSTRACT

Purpose: To report a case of *Acanthamoeba* keratitis diagnosed using confocal microscopy in a patient corrected by orthokeratology and treated with corneal crosslinking (CXL) after failure to respond to medical treatment.
Methods: After diagnosis, the patient was treated with several medications until CXL was applied during one 30-min session using ultraviolet A radiation and application of riboflavin. The clinical signs of the disease observed using slit-lamp biomicroscopy and confocal microscopy were evaluated and the visual acuity was measured during the course of the infection and treatment over a period of 30 months including 12 months of medical treatment, 9 months after cross-linking and amniotic membrane transplant and 9 months after penetrating keratoplasty and cataract extraction.
Results: In this case, confocal microscopy facilitated early diagnosis of an *Acanthamoeba* infection even if other signs and symptoms might be confounding. CXL was more effective than aggressive medication against the microorganism. After CXL, the symptoms and the corneal appearance improved significantly but the ulcer did not heal completely. After amniotic membrane transplantation, the patient underwent penetrating keratoplasty (PK) with no rejection, and the visual function substantially improved over 9 months of follow-up.
Conclusions: Swimming in contaminated water might represent a risk for orthokeratology patients. CXL was effective for treating *Acanthamoeba* keratitis in an orthokeratology patient to eliminate active and cystic forms of the microorganism. Confocal microscopy was useful to confirm the diagnosis in the presence of confounding clinical signs observed during a conventional slit-lamp examination. Both CXL and confocal microscopy are essential to the outcome of PK.

1. Introduction

Acanthamoeba species (spp.), is a unicellular protozoa capable of forming amoeba cysts, are present in air, soil, and fresh water (baths, swimming pools, and stagnant water). In humans they are among the normal oral flora. They are resistant to many disinfecting agents, changes in temperature, and dry environments. The first case of an *Acanthamoeba* ocular infection was reported in 1974 [1]. Since then many cases have been reported, most of which were unilateral. Binocular cases also have been described. In non-contact lens wearers, infection traditionally has been associated with previous trauma and subsequent exposure to contaminated water or soil [2]. This infection is also commonly associated with contact lens wear, particularly disposable soft contact lenses [3,4]. *Acanthamoeba* has demonstrated to adhere to rigid, rigid gas permeable and soft contact lenses and it has been hypothesized as a potential risk for infection [5,6]. In the literature we can find *Acanthamoeba* infection associated also to rigid gas permeable lenses for orthokeratology [7] and also to hybrid lenses [8]. *Acanthamoeba* infection related with contact lens wear use to be associated with poor compliance or contact with contaminated water sources [9,10]. *Acanthamoeba* has been associated to one of the more recent contact lens related infections outbreaks in the USA [11], and continuous efforts are being done by care solutions manufacturers to increase the efficacy against this resistant microorganism [12–14].

☆ The authors have no proprietary or financial interest in any of the materials mentioned in this article.

* Corresponding author at: Clinical & Experimental Optometry Research Lab, Department of Physics (Optometry), University of Minho, 4710-057 Braga, Portugal. Tel.: +351 253 60 43 20; fax: +351 253 67 89 81.
E-mail address: jgmeijome@fisica.uminho.pt (J.M. González-Méijome).

1367-0484/$ – see front matter © 2013 British Contact Lens Association. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.clae.2013.11.008

The pathophysiology of *Acanthamoeba* infection includes severe corneal necrosis; establishing the diagnosis is usually difficult and results in delayed administration of appropriate treatment. The outcomes can range from partial loss of corneal transparency to corneal transplant or even enucleation. The differential diagnosis includes herpes simplex, fungal keratitis, or torpid evolution keratitis [15]. Early treatment is essential for appropriate management, and confocal microscopy is a powerful tool in this regard [15].

In 2003, Wollensak et al. [16] introduced corneal cross-linking (CXL), which consists of application of ultraviolet-A (UVA) radiation onto the cornea after epithelial debridement and irrigation with isomolar riboflavin solution at 0.1% at regular intervals to cross-link the corneal stroma and strengthen the corneal tissue. CXL is effective for stabilizing keratoconus and post-refractive surgery ecstatia [17]. In vitro studies also have shown the bactericidal effect of UV radiation, which seems to be enhanced with riboflavin. The treatment is effective against *Staphylococcus aureus*, methicillin-resistant *S. aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, multiresistant *P. aeruginosa*, and drug-resistant *Streptococcus pneumonia* [18]. More recently, the combined application of amphotericin B and riboflavin with UV radiation was effective against *Candida albicans*, *Fusarium* spp., and *Aspergillus fumigatus* [19].

2. Case report

A 34-year-old woman, who had been wearing corneal refractive therapy lenses for myopia correction for 3 years, presented with the complaints of tearing, burning, photophobia, and moderate pain of 4 days duration after having bathed in a swimming pool that was poorly maintained. The patient worn the current lenses for 9 months at the time of the event and was compliant with the care regime consisting of a multipurpose care solution for gas permeable lenses (Boston Simplus, Bausch & Lomb), a monthly protein removal (Progent, Menicon) and preservative free artificial tears to apply with lens insertion and immediately after waking every morning (Aquify, Ciba Vision). When she was informed that she might have an ocular infection, the patient spontaneously reported that other users of the swimming pool she used to attend had recently developed ocular infections and other adverse events potentially related to the quality of the pool water.

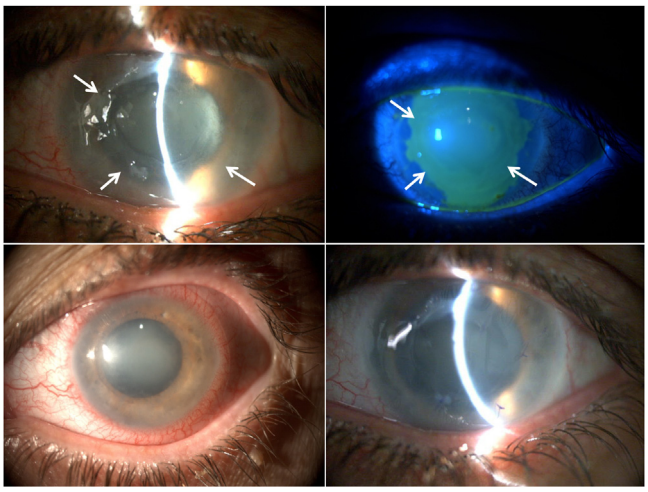

Fig. 1. Ulcer formation and follow-up after CXL treatment. Top left, the ulcer after CXL treatment; top right, the ulcer after CXL stained with sodium fluorescein; bottom left, progression of the ulcer after CXL covered with a therapeutic contact lens; bottom right, after an amniotic transplantation procedure. Images obtained at 10× (top right) and 12× magnification.

Slit-lamp examination confirmed a mild inferior superficial keratitis. Considering the suspicion of an inflammatory adverse event, a topical antibiotic (Tobrex, Tobramycin, Alcon, Spain) (1 drop, 4 times/day) was prescribed, and cessation of contact lens wear was recommended until resolution of the infection. After 5 days of treatment, the pain and photophobia increased, and an examination revealed infiltration with a dendritic fluorescein pattern compatible with a herpetic infection. Acyclovir ointment (GlaxoSmithKline, Spain) five times daily was prescribed. The lack of improvement within the next 72 h and increasing pain led us to consider the possibility of *Acanthamoeba* keratitis. Suspect of polymicrobial infection was considered and epithelial specimens were collected for culture assays of bacteria, fungi, and *Acanthamoeba*. The solution in the contact lens case also was analyzed. The diagnosis of *Acanthamoeba* was confirmed by confocal microscopy (Fig. 1) based on the presence of subepithelial cysts [20,21]. All other laboratory cultures were negative.

Treatment consisting of topical chlorhexidine (0.02%), polymyxin, neomycin, gramicidin (Ophthalmowell, Farmasierra Manufacturing SL, Madrid, Spain), and propamidine isethionate 0.1% (Brolene, Sanofi Aventis, Surrey, UK); the medications were instilled hourly during the day and every 4 h at night. Cyclopentolate 0.1% (Colircusi Cicloplejico, Alcon, Barcelona, Spain) was prescribed every 8 h and systemic ketoconazole (Panfungol, Laboratorios Dr. Esteve SA, Barcelona) 100 mg every 12 h.

After a few weeks of treatment, perineuritis was present, and the typical ring-shaped infiltrate characteristic of *Acanthamoeba* keratitis was observed at a later stage.

After 4 months of intensive treatment with no substantial improvement, propamidine isethionate 0.1% was replaced with hexamidine 0.1% (Desomedine, Pharma Intenracional, Spain), and chlorhexidine 0.02% was replaced with polyhexamethylene biguanide (PHMB) from Moorfields Eye Hospital, London, UK.

After 1 year, the corneal conditions worsened with ulceration and recurrent uveitis, and CXL was performed. Topical anesthesia of the cornea was obtained using oxybuprocaine and tetracaine drops, alternating every 3 min for 9 min. During this time period, pilocarpine 2% eye drops were also instilled twice. After a lid speculum was inserted, a 9.0-mm diameter corneal abrasion was created. Ribofl avin 0.1% drops were instilled every 3 min for 30 min. The ribofl avin drops were prepared immediately before treatment by mixing aqueous ribofl avin 0.5% solution with dextran T-500 20% solution. UVA radiation was applied at a rate of 3 mW/cm^2 with a CMB XLINKER (Ophthaltec, Barcelona, Spain), and riboflavin solution was applied every 5 min for 30 min. During irradiation, the cornea was moistened every 5 min with ribofl avin 0.1% drops and oxybuprocaine drops every 10 min.

After CXL, the corneal appearance improved significantly and the symptoms resolved. The signs of inflammation also resolved almost immediately, although *Acanthamoeba* treatment was interrupted only 3 months later. Confocal microscopy at this stage showed no cysts in the area of analysis.

Six months after CXL, amniotic membrane implantation was performed to resolve the deficient corneal re-epithelization. Fig. 2 shows the ocular condition at different time points after CXL.

After this stage, corneal melting developed about 8 months after CXL and 20 months after first onset of the infection with resultant glaucoma and a triple procedure that included glaucoma and cataract surgeries and penetrating keratoplasty (PK) was scheduled. The surgeries were performed when no *Acanthamoeba* cysts were seen on confocal microscopy. The triple procedure was uneventful and the patient has had no signs of rejection of the implant or other complications. Fig. 3 shows the current appearance of the eye with recovery of the best-corrected visual acuity to 20/60.

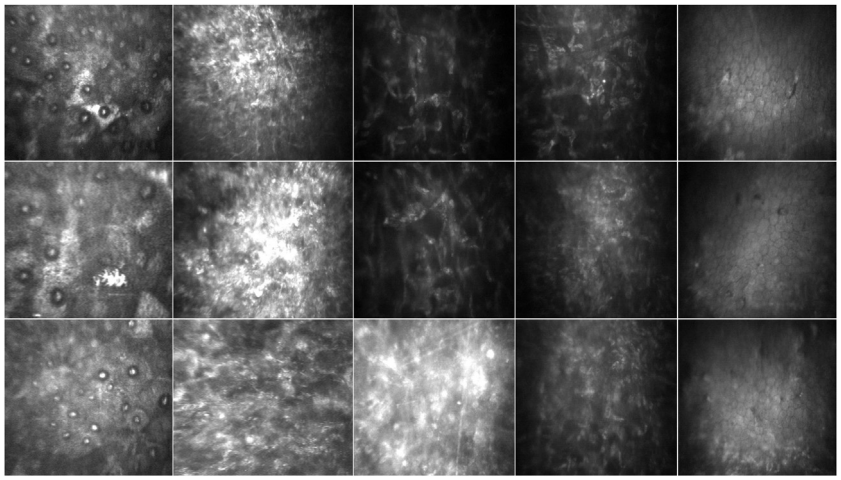

Fig. 2. Confocal micrographs of the corneal epithelium (left side), anterior, medium and posterior stroma, and endothelium (right side) in the superior cornea (top row), central cornea (medium row) and inferior cornea (bottom row). Bright circular bodies in the epithelial region indicate the presence of *Acanthamoeba* cysts. The stromal micrographs depict a highly disorganized structure in the anterior stroma affected by the infection and a progressively less hyper reflective aspect at deeper locations. Hexagonal cell pattern is observed in the endothelial layer, particularly in the superior and central location.

3. Discussion

Fortunately, *Acanthamoeba* keratitis is rare, but clinicians must consider it in the differential diagnosis, particularly in patients who wear contact lenses and present with pain that is incompatible with inflammation and/or an epithelial defect. Suspects of non-compliance with care regimes, use of tap water to rinse contact lenses or exposure to potentially contaminated sources of water are relevant to suspect the presence the infection. Swimming in lenses is considered a risk factor for *Acanthamoeba* eye infection [22]. Orthokeratology represents a different situation in which the lenses are not work during the water exposure as the lenses are worn overnight. New techniques allow now an earlier identification of *Acanthamoeba* infection [23–25], but diagnosis is still difficult and most cases of *Acanthamoeba* keratitis are misdiagnosed initially as herpetic keratitis and less frequently as bacterial or fungal keratitis because the symptoms and sometimes the signs are non-specific [26]. Care should be taken with negative cultures for bacteria and fungi that do not improve with medical treatment. Confocal microscopy was useful to confirm the diagnosis considering that the *Acanthamoeba* culture is negative in most cases [20,24]. Early diagnosis and appropriate treatment are essential for a good outcome in these infections and physicians need to be aware of history of contact lens wear or other suspicious signs as dendritic keratitis [15]. In this case, despite the relatively early treatment (15 days after the first visit and about 20 days after the initial symptoms), the final outcome was unsatisfactory. Treatment included the drugs of choice for these cases (diamine and biguanide-derived drugs) neomycin to avoid induction of bacterial infection, and a cycloplegic agent to relieve pain due to iris spasms and prevent synechiae. Oral antifungal drugs 200 mg every 12 h also have been effective against amoeba [27].

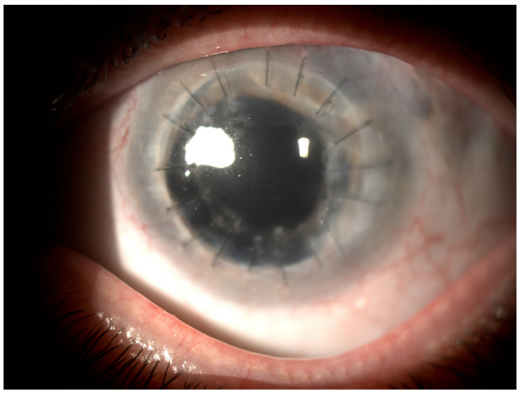

Fig. 3. Final outcome after penetrating keratoplasty with a best spectacle corrected visual acuity of 20/60. Diffuse observation at 12× magnification.

Considering the possibility of the presence of propamidine-resistant *Acanthamoeba* strains, a change in medication also was considered in this case. PHMB has been effective against the cystic form of *Acanthamoeba* even at low concentrations [28,29]. Despite these considerations, the different medical strategies failed, so a conservative surgical solution was considered rather than a penetrating corneal transplant. In addition, penetrating keratoplasty was not possible while the infection was active. Additional suspects of polymicrobial infection were also considered at this point.

The use of UV light as an antimicrobial agent has been long recognized. However, recently the benefit of the combined application of UV light and riboflavin-A in CXL was found to be more effective than UV light alone against different strains of bacteria such as *S. epidermidis*, *S. aureus*, and *P. aeruginosa* [30]. The current report is not the first on the use of CXL against the collagenolytic activity of microorganisms such as *Acanthamoeba*. Moren et al. performed CXL to treat a case of presumed *Acanthamoeba* infection in a contact lens wearer with good wound healing and visual rehabilitation [31]. Conversely, Rama et al. were unsuccessful when performing CXL in a patient with keratoconus who developed corneal melting with a positive culture for *Acanthamoeba* [32]; corneal perforation developed 5 days after treatment. The effectiveness of CXL also may be limited by the depth of the treatment, which usually is limited to the anterior 300 μm. If the microorganisms penetrate below this level, the treatment might be unsuccessful. Other cases have been also recently described in the literature although none was related with orthokeratology lenses.

To the best of our knowledge, the current study is the first to report the use of CXL to treat an infection caused by corneal refractive therapy. The safety of orthokeratology has been questioned after several cases of microbial keratitis developed in children [7,33], and a review of the potential risks was published recently by the American Academy of Ophthalmology [34]. Nevertheless, most cases of infection reported until now presumably were associated with potentially risky practices such as poor contact lens

care systems. The current patient reported swimming daily and that, at the time of diagnosis, other swimmers in the same pool had developed ocular infections. The use of lenses in this environment has been recognized as a potential risk for ocular infection [22,35]. Despite orthokeratology lenses are not worn while swimming, *Acanthamoeba* keratitis should not be ruled out in these patients as the organisms might also be present on the eye when the lenses are wearing, and this might increase the risk of corneal attachment and infection as these lenses have been relates with higher retention of other bacteria as *P. aeruginosa* [36]. Some studies have reported similar or higher adhesion of *Acanthamoeba* to gas permeable and soft contact lens materials [5,6]. Due to the close relationship between the cornea and orthokeratology lenses, this might be a potential risk [36]. Early diagnosis and aggressive treatment are the approaches in such cases. However, when the patient does not respond to treatment, other alternatives such as CXL might be considered. Confocal microscopy has been useful for diagnosis and for ensuring that the cornea is free of residual infection in the form of trophozoites or cysts before proceeding with penetrating keratoplasty.

In summary, the use of confocal microscopy was essential in the current case to establish the diagnosis and initiate an early specific treatment. The use of CXL, although it seems to be effective in stopping the progress of the infection, cannot always result in a successful definitive treatment. Furthermore, patients corrected with orthokeratology should be warned that under certain adverse conditions a contaminated swimming pool may pose a potential risk of severe adverse events.

References

[1] Naginton J, Watson PG, Playfair TJ, Mc Gill J, Jones BR, Steele AD. Amoebic infection of the eye. Lancet 1974;2:1537–40.
[2] Illingworth CD, Cook SD. *Acanthamoeba* keratitis. Surv Ophthalmol 1998;42:493–508.
[3] Sadiq SA, Azuara-Blanco A, Bennett D, Lloyd JH, Dua HS. Evaluation of contamination of used disposable contact lenses by *Acanthamoeba*. CLAO J 1998;24:155–8.
[4] Ficker L, Hunter P, Seal D, Wright P. *Acanthamoeba* keratitis occurring with disposable contact lens wear. Am J Ophthalmol 1989;108:453.
[5] Sharma S, Ramachandran L, Rao GN. Adherence of cysts and trophozoites of *Acanthamoeba* to unworn rigid gas permeable and soft contact lenses. CLAO J 1995;21:247–51.
[6] Kelly LD, Long D, Mitra D. Quantitative comparison of *Acanthamoeba castellanii* adherence to rigid versus soft contact lenses. CLAO J 1995;21:111–3.
[7] Watt KG, Swarbrick HA. Trends in microbial keratitis associated with orthokeratology. Eye Contact Lens 2007;33:373–7.
[8] Lee WB, Gotay A. Bilateral *Acanthamoeba* keratitis in Synergeyes contact lens wear: clinical and confocal microscopy findings. Eye Contact Lens 2010;36:164–9.
[9] Pens CJ, da CM, Fadanelli C, Caumok K, Rott M. *Acanthamoeba* spp. and bacterial contamination in contact lens storage cases and the relationship to user profiles. Parasitol Res 2008;103:1241–5.
[10] Kilvington S, Gray J, Morlet N, Beeching JR, Frazer DG, Matheson M. *Acanthamoeba* keratitis: the role of domestic tap water contamination in the United Kingdom. Invest Ophthalmol Vis Sci 2004;45:165–9.
[11] Tu EY, Joslin CE. Recent outbreaks of atypical contact lens-related keratitis: what have we learned? Am J Ophthalmol 2010;150:602–8.
[12] Uno T, Ohashi Y, Nomachi M, Imayasu M. Effects of multipurpose contact lens care solutions on the adhesion of *Acanthamoeba* to silicone hydrogel contact lenses. Cornea 2012;31:1170–5.
[13] Kilvington S, Huang L, Kao E, Powell CH. Development of a new contact lens multipurpose solution: comparative analysis of microbiological, biological and clinical performance. J Optom 2010;3:134–42.
[14] Borazjani RN, Kilvington S. Efficacy of multipurpose solutions against *Acanthamoeba* species. Cont Lens Anterior Eye 2005;28:169–75.
[15] Hammersmith KM. Diagnosis and management of *Acanthamoeba* keratitis. Curr Opin Ophthalmol 2006;17:327–31.
[16] Wollensak G, Spoerl E, Seiler T. Riboflavin/ultraviolet-a-induced collagen crosslinking for the treatment of keratoconus. Am J Ophthalmol 2003;135:620–7.
[17] Wollensak G, Iomdina E. Long-term biomechanical properties of rabbit cornea after photodynamic collagen crosslinking. Acta Ophthalmol 2009;87:48–51.
[18] Martins SA, Combs JC, Noguera G, Camacho W, Wittmann P, Wather 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.
[19] Sauer A, Letscher-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.
[20] Mathers WD, Nelson SE, Lane JL, Wilson ME, Allen RC, Folberg R. Confirmation of confocal microscopy diagnosis of *Acanthamoeba* keratitis using polymerase chain reaction analysis. Arch Ophthalmol 2000;118:178–83.
[21] Kanavi MR, Javadi M, Yazdani S, Mirdehghanm S. Sensitivity and specificity of confocal scan in the diagnosis of infectious keratitis. Cornea 2007;26:782–6.
[22] Choo J, Vuu K, Bergenske P, Burnham K, Smythe J, Caroline P. Bacterial populations on silicone hydrogel and hydrogel contact lenses after swimming in a chlorinated pool. Optom Vis Sci 2005;82:134–7.
[23] Chew HF, Yildiz EH, Hammersmith KM, Eagle Jr RC, Rapuano CJ, Laibson PR, et al. Clinical outcomes and prognostic factors associated with *Acanthamoeba* keratitis. Cornea 2011;30:435–41.
[24] Vaddavalli PK, Garg P, Sharma S, Rao GN, Thomas R. Role of confocal microscopy in the diagnosis of fungal and *Acanthamoeba* keratitis. Ophthalmology 2011;118:29–35.
[25] Shah SG, Sharma S, Fernandes M, Lakshmipathy M. Rapid detection of *Acanthamoeba* cysts in frozen sections of corneal scrapings with Fungiflora Y. Br J Ophthalmol 2010;94:1550–1.
[26] Hsieh WC, Dornic DI. *Acanthamoeba* dendriform keratitis. J Am Optom Assoc 1989;60:32–4.
[27] Visvesvara GS. Amebic meningoencephalitides and keratitis: challenges in diagnosis and treatment. Curr Opin Infect Dis 2010;23:590–4.
[28] Lin HC, Hsiao CH, Ma DH, Yeh LK, Tan HY, Lin MY, et al. Medical treatment for combined *Fusarium* and *Acanthamoeba* keratitis. Acta Ophthalmol 2009;87:199–203.
[29] Lim N, Goh D, Bunce C, Xing W, Fraenkel G, Poole TR, et al. Comparison of polyhexamethylene biguanide and chlorhexidine as monotherapy agents in the treatment of *Acanthamoeba* keratitis. Am J Ophthalmol 2008;145:130–5.
[30] Makdoumi K, Backman 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.
[31] Moren H, Malmsjo M, Mortensen J, Ohrstrom A. Riboflavin and ultraviolet a collagen crosslinking of the cornea for the treatment of keratitis. Cornea 2010;29:102–4.
[32] Rama P, Di MF, Matuska S, Paganoni G, Spinelli A. *Acanthamoeba* keratitis with perforation after corneal crosslinking and bandage contact lens use. J Cataract Refract Surg 2009;35:788–91.
[33] Watt K, Swarbrick HA. Microbial keratitis in overnight orthokeratology: review of the first 50 cases. Eye Contact Lens 2005;31:201–8.
[34] Van Meter WS, Musch DC, Jacobs DS, Kaufman SC, Reinhart WJ, Udell IJ. Safety of overnight orthokeratology for myopia: a report by the American Academy of Ophthalmology. Ophthalmology 2008;115:2301–13.
[35] Radford CF, Minassian DC, Dart JK. *Acanthamoeba* keratitis in England and Wales: incidence, outcome, and risk factors. Br J Ophthalmol 2002;86:536–42.
[36] Choo JD, Holden BA, Papas EB, Willcox MD. Adhesion of *Pseudomonas aeruginosa* to orthokeratology and alignment lenses. Optom Vis Sci 2009;86:93–7.