Use of white light in vivo confocal microscopy for the detection of spatial changes in the corneal nerves in cases of early-stage Acanthamoeba keratitis with radial keratoneuritis

Kuo-Chi Hung\textsuperscript{1,2,3}, Chia-Ju Lu\textsuperscript{1,3}, Hsin-Yu Liu\textsuperscript{1}, Yu-Chih Hou\textsuperscript{1}, I-Jong Wang\textsuperscript{1}, Fung-Rong Hu\textsuperscript{1,3}, Wei-Li Chen\textsuperscript{1,3}

**Purpose:** Radial keratoneuritis (RK) is a common feature of Acanthamoeba keratitis (AK). In vivo confocal microscopy (IVCM) is noninvasive and provides real-time images for the diagnosis of corneal diseases by allowing the visualization of corneal structures and morphologies of living organisms at the cellular level. Images of AK with RK obtained using commercial white light IVCM devices have not been frequently evaluated. In the present study, a white light IVCM device was used to evaluate the corneal findings and describe spatial changes in the corneal nerves at different depths in cases of early-stage AK with RK.

**Methods:** In this retrospective, observational study, white light IVCM images focused on RK were evaluated for Acanthamoeba cysts/trophozoites, corneal deposits, and altered corneal nerves, with special emphasis on three-dimensional spatial changes in the corneal nerves at different depths. **Results:** Seventeen eyes of 17 patients exhibiting early-stage AK with RK were included in the study. Acanthamoeba cysts/trophozoites were observed in the corneal epithelium of 13 eyes and stroma of 7 eyes. Alterations in the corneal nerve morphology and density were observed from the basal epithelial layer to the stromal layer in 12 eyes. Acanthamoeba trophozoites were attached to the corneal stromal nerves in five eyes. **Conclusion:** These findings suggest that white light IVCM can identify consistent corneal findings, particularly spatial changes in the corneal nerves, in cases of early-stage AK with RK.

**Key words:** Acanthamoeba keratitis, corneal nerves, in vivo confocal microscopy, radial keratoneuritis, trophozoites

Acanthamoeba keratitis (AK) is a vision-threatening infectious disease caused by Acanthamoeba protozoa.\cite{1}\cite{2}\cite{3} After reviewing our past medical records, we incidentally discovered that spatial changes in the corneal nerves in some cases of early-stage AK with Radial keratoneuritis (RK). In the present study, we reviewed our past medical records. White light in vivo confocal microscopy (IVCM) was used to assess cases of early-stage AK with RK. Images were obtained at different corneal layers and assessed for the characteristic findings of brightly reflective abnormal cells, corneal deposits, and altered corneal nerve structures, with special focus on spatial changes in the corneal nerves at different depths. The anatomical relationship between the Acanthamoeba pathogens and the abnormal corneal nerves was also evaluated.

**Methods**

This retrospective, cross-sectional study included consecutive patients diagnosed with early-stage AK between May 2008 and April 2016. Informed consent was obtained from all subjects after the nature, purpose, and possible consequences of the study were explained. The appropriate ethics committee approved the research protocol and the methods used in the study adhered to the tenets of the Declaration of Helsinki for the use of human subjects in biomedical research. Seventeen eyes of 17 patients were included in this study. All patients wore contact lenses, with 13 wearing soft contact lenses and four wearing orthokeratology lenses. Corneal epithelium specimens were sent for smear and culture. The data on ophthalmological history, best-corrected visual acuity (BCVA), intraocular pressure, and slit-lamp biomicroscopic findings were collected from chart reviews.

**In vivo confocal microscopy**

IVCM was performed for all patients by a single examiner at the first visit. One drop of 0.5% proparacaine solution and one drop of artificial tears were instilled just before the examination. A white light IVCM device (Confoscan 3.4.1; Nidek Technologies) equipped with a standard 40× water-immersion front lens (Zeiss, Oberkochen, Germany) captured the full

\textsuperscript{1}Department of Ophthalmology, National Taiwan University Hospital,\textsuperscript{2}Center of Corneal Tissue Engineering and Stem Cell Biology, National Taiwan University, Taipei,\textsuperscript{3}Department of Ophthalmology, Sinying Hospital, Ministry of Health and Welfare, Xinying, Tainan, Taiwan

\textsuperscript{1}The first two authors have contributed equally to this work

**Correspondence to:** Dr. Wei-Li Chen, Department of Ophthalmology, National Taiwan University Hospital, 7 Chung-Shan South Road, 10002, Taipei, Taiwan. E-mail: chenwei@ntu.edu.tw

Received: 16-Jul-2019 Revised: 03-Oct-2019 Accepted: 11-Dec-2019 Published: 25-May-2020

© 2020 Indian Journal of Ophthalmology | Published by Wolters Kluwer - Medknow

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

**For reprints contact:** reprints@medknow.com

Cite this article as: Hung KC, Lu CJ, Liu HY, Hou YC, Wang IJ, Hu FR, et al. Use of white light in vivo confocal microscopy for the detection of spatial changes in the corneal nerves in cases of early-stage Acanthamoeba keratitis with radial keratoneuritis. Indian J Ophthalmol 2020;68:1061-6.
thickness of the central cornea using an automatic capture technique. Special attention was paid to RK visualization. Each examination required approximately 1 to 3 min and recorded 350 images. During each visit, the measurements were repeated three times with a 4-µm z-interval, followed by three times with a 1.5-µm z-interval.

Results

Demographics
Four men and 13 women (mean age, 20.8 ± 6.4 years) were diagnosed with early-stage AK with RK [Fig. 1]. All patients wore contact lenses, with 13 wearing soft contact lenses and four wearing orthokeratology lenses. The mean duration from onset to the initial visit was 27.2 ± 3.4 days. All cultures and smears had shown positive findings for AK. All patients were treated with oral and topical anti-amoebic agents and cured without antibiotics, anti-fungal medications, or surgical management.

In vivo confocal microscopy

Findings in the corneal epithelial layer
In the basal epithelial layer, *Acanthamoeba* cysts were observed in 10 eyes as round, hyper-reflective particles with a diameter of 10 to 20 µm [Fig. 2a], while *Acanthamoeba* trophozoites were observed in 10 eyes as amorphous, hyper-reflective, irregular, wedge-shaped structures with a diameter of 10 to 20 µm. *Acanthamoeba* pathogens in the basal epithelial layer were observed without [Fig. 2a] or with [Fig. 2b and c] attachment to the basal epithelial nerves. Morphological changes in the corneal nerves in this layer or Bowman’s layer were observed in eight eyes. These corneal nerves appeared thin, fragmented, and tortuous [Fig. 2c and d], with no changes in their diameters.

Findings in the corneal stromal layer
In the corneal stromal layer, *Acanthamoeba* cysts were observed in five eyes as round, hyper-reflective particles with a diameter of 10 to 20 µm [Fig. 2e and f]. Hyper-reflective spindle-shaped structures in the stroma were found in four eyes [Fig. 2f], while hyper-reflective, activated keratocytes forming a honeycomb pattern were found in 10 eyes [Fig. 2g].

Changes in the corneal nerves in the stroma
Changes in the corneal nerve structure exhibited two different patterns. In two eyes, thin, tortuous, fragmented corneal nerves with no significant changes in their diameters were observed from the corneal basal epithelial layer to the corneal stroma [Fig. 2c, d and h]. In five eyes, on the other hand, the thin,
tortuous, fragmented corneal nerves in the basal epithelial layer exhibited increased diameter and density and were mostly distributed parallel to one another [Fig. 2h] after the penetration of Bowman’s membrane. Amoeba trophozoites were suspected to be attached to the intrastromal nerve trunks [Fig. 2i]. Table 1 summarizes the above IVCM findings, and Table 2 summarizes the sensitivity of the IVCM findings for the diagnosis of AK from corneal scrapes.

**Representative case**

A 31-year-old woman who wore soft contact lenses for refractive correction in both eyes presented with a complaint of ocular discomfort in the left eye for 2 weeks. The BCVA for the left eye was 20/25. Slit-lamp biomicroscopy showed severe conjunctival infection with numerous swollen corneal nerves that were characteristic of RK emerging from the limbus. IVCM images showed *Acanthamoeba* cysts in the basal epithelial layer [Fig. 3a and b]. A diagnosis of AK with RK was also confirmed by culture positivity for *Acanthamoeba*. A combination of topical polyhexamethylene biguanide, chlorhexidine, propamidine, and clotrimazole was applied hourly. The patient recovered after treatment and remained stable without recurrence.

**Table 1: IVCM findings of 17 eyes (17 patients) diagnosed with early-stage AK with RK**

| Case No. | Sex/ Age | Surface epithelium | Basal epithelium | Stroma | Sensitivity of in vivo confocal microscopy for the detection of AK (%) |
|----------|----------|--------------------|------------------|--------|---------------------------------------------------------------|
|          |          |                    |                  |        |                                                               |
| 1        | F/12     | +                  | +                | +      | 58.82                                                         |
| 2        | F/13     | +                  | +                | +      | 58.82                                                         |
| 3        | F/14     | +                  |                  | +      |                                                               |
| 4        | F/15     | +                  | +                | +      |                                                               |
| 5        | F/15     | +                  | +                | +      |                                                               |
| 6        | F/17     | +                  | +                | +      |                                                               |
| 7        | F/17     | +                  |                  | +      |                                                               |
| 8        | F/22     | +                  |                  | +      |                                                               |
| 9        | F/24     | +                  | +                | +      |                                                               |
| 10       | F/27     | +                  | +                | +      |                                                               |
| 11       | F/31     | +                  | +                | +      |                                                               |
| 12       | F/34     | +                  |                  | +      |                                                               |
| 13       | F/24     | +                  | +                | +      |                                                               |
| 14       | M/19     | +                  |                  | +      |                                                               |
| 15       | M/20     | +                  |                  | +      |                                                               |
| 16       | M/22     | +                  |                  | +      |                                                               |
| 17       | M/26     | +                  |                  | +      |                                                               |
| Total    |          | 10/17              | 8/17             | 5/17   | 5/17                                                         |

*Acanthamoeba* cysts and trophozoites were observed in the basal epithelial layer in 10 of 17 eyes. Morphological changes in corneal nerves in the basal epithelial layer or Bowman’s layer were found in 8 of 17 eyes. In the corneal stromal layer, *Acanthamoeba* cysts were observed in 5 of 17 eyes. Highly reflective, spindle-shaped materials in the stroma were found in 4 of 17 eyes. Activated keratocytes forming a honeycomb pattern were found in 10 of 17 eyes. Thin, tortuous, and fragmented stromal nerves with no change in corneal diameter and were found in 2 of 17 eyes. Five of 17 eyes showed thickened stromal nerves with increased nerve density. IVCM=In vivo confocal microscopy, AK=*Acanthamoeba* keratitis, RK=Radial keratoneuritis

**Table 2: Pathological IVCM findings in 17 patients with early-stage AK with RK**

| Corneal Sublayer | Finding                  | Number of Patients | Sensitivity of in vivo confocal microscopy for the detection of AK (%) |
|------------------|--------------------------|--------------------|---------------------------------------------------------------|
|                  |                          |                    |                                                               |
| Surface epithelium | *Acanthamoeba* cysts     | 10                 | 58.82                                                         |
|                  | *Acanthamoeba* trophozoites | 10                 | 58.82                                                         |
| Basal epithelium | Nerve changes             | 8                  | 47.06                                                         |
| Stroma           | *Acanthamoeba* cysts     | 5                  | 29.41                                                         |
|                  | *Acanthamoeba* trophozoites | 5                  | 29.41                                                         |
|                  | Honeycomb patterns       | 10                 | 58.82                                                         |
|                  | Spindle-shaped structures | 4                  | 23.53                                                         |
|                  | Thickened nerves with increased density | 5 | 29.41                                                         |
|                  | Thin, tortuous nerves    | 2                  | 11.76                                                         |
|                  | Inflammatory cells       | 17                 | 100.00                                                        |

IVCM=In vivo confocal microscopy, AK=*Acanthamoeba* keratitis, RK=Radial keratoneuritis
Serial IVCM images clearly showed spatial changes in the corneal nerves. The basal epithelial layer [Fig. 3a] showed thin, fragmented corneal epithelial nerves with a few highly reflective, coffee bean-shaped particles that were strongly suspected to be *Acanthamoeba* cysts. The deeper layer of corneal nerves near Bowman’s layer appeared engorged and tortuous [Fig. 3b]. The diameter of the corneal nerves had increased, and the nerves became more tortuous as deeper layers of the corneal stroma were examined [Fig. 3c]. In the deeper layers, the density of the thickened corneal stromal nerves had also increased. Strongly hyper-reflective, irregular, wedge-shaped structures suspected to be *Acanthamoeba* trophozoites were found along the corneal nerves [Fig. 3d]. The stromal nerves showed a continuous increase in their size, tortuosity, and density with an increase in depth in the middle stroma. Hyper-reflective, irregular, wedge-shaped structures that were strongly suspected to be *Acanthamoeba* trophozoites were still attached to the middle stromal nerves [Fig. 3e and f].

**Discussion**

To the authors’ knowledge, this is the largest case series using IVCM for the detection of early-stage AK with RK and evaluation of spatial changes in the corneal nerves at different depths. Instead of analyzing single cross-sectional images, the nerves on serial images were assessed to account for their three-dimensional structures. The present findings suggested that white light IVCM could identify consistent corneal findings, particularly spatial changes in the corneal nerves, in cases of early-stage AK with RK.

IVCM has been found to exhibit high sensitivity and specificity for AK detection and it can provide information that microbiological tests such as smear and culture cannot.[2-7] There are two major types of IVCM devices in the market:
laser (e.g., HRT; Heidelberg Engineering GmbH) and white light (e.g., Confoscan; Nidek Technologies). Although both HRT and white light IVCAM are widely available on the market and widely used in the clinic, they have different features and technical specifications.[8] Confoscan is superior in that it is equipped with an automatic operating mode, which is lacking in HRT. This feature is very convenient for use in the busy clinic and by inexperienced examiners, as it can provide good-quality images within the short examination time and has a short learning curve. Besides, white light IVCAM does not have the disadvantage of possible lesion-like artifacts in imaging corneas (e.g., stromal striae and Descemet’s membrane folds), which are frequently found in HRT IVCAM because of its direct microscope application to the cornea.

These two systems have different optical designs and imaging qualities. Confoscan is equipped with a ×40 front lens (Zeiss). Manual, semi-automatic, and automatic operating modes allow the production of back and forth movements during image acquisition. The HRT laser is equipped with a ×60 front lens (Olympus, Tokyo, Japan) and employs a manual technique for imaging of the entire corneal thickness; a semi-automatic technique covers only the anterior 85 µm. Therefore, the detection of larger or deeper areas may be more difficult with laser IVCAM than with white light IVCAM, which is especially important for clinical use, as IVCAM examination is mostly needed for clinical conditions with corneal endothelial manifestations.

RK is considered an early pathognomonic presentation of AK.[9-12] Several studies, including the present one, have used IVCAM to detect Acanthamoeba cysts and trophozoites, the honeycomb pattern of active keratoocytes, infiltration of inflammatory cells, and highly reflective spindle-shaped structures representative of RK.[6,9] The present study showed that Acanthamoeba cysts and trophozoites can be observed in the basal epithelial and stromal layers [Figs. 2 and 3]. Acanthamoeba cysts were noted as hyper-reflective, round particles with a diameter of 10 to 20 µm, and Acanthamoeba trophozoites were observed to be amorphous, hyper-reflective, bright, irregular, wedge-shaped structures with a diameter of 10 to 20 µm.[8,14] These morphometric findings were typical and consistent with those of a previous study conducted by Kanavi et al.[15]

The mechanism underlying the development of RK in eyes with early-stage AK is not fully understood. Most clinicians and researchers believe that RK is caused by an immune reaction to amoebic migration into the corneal stroma and along the corneal stromal nerves.[12,15] It has been surmised that both nerve invasion by trophozoites and the resultant swelling may cause severe pain associated with AK. It has also been suggested that Acanthamoeba trophozoites track along and feed on the corneal nerves.[8] A study by Kobayashi et al. used the HRT laser IVCAM system to detect corneal pathological changes in 13 eyes with RK[9] and found that Acanthamoeba cysts existed almost exclusively in the basal epithelial cell layer. No stromal or nerve infiltration by Acanthamoeba cysts and trophozoites was found and it was concluded that RK may be mediated by inflammatory molecules such as cytokines that are released by the Acanthamoeba trophectin in the corneal epithelium. Immune or inflammatory mediators may diffuse through the corneal stroma into the corneal nerves or affect corneal nerve endings in the epithelial cell layer. In the present study, seven of 17 eyes exhibited Acanthamoeba cysts or trophozoites in the corneal stroma, with some of these cysts/trophozoites attached to the corneal nerves [Fig. 2e, f and i]. This finding suggests that Acanthamoeba cysts and trophozoites have already penetrated the corneal stroma in the early stages of AK with RK. The development of RK may be partially attributed to a direct attack of the corneal nerves by Acanthamoeba.

Alterations in the morphology and density of the corneal nerves in early-stage, late-stage, and cured AK have been found in studies using laser IVCAM.[17-19] In these studies, the corneal nerve density and length were found to have decreased in the acute stage of AK, followed by regeneration in later stages. In contrast, a study by Kanavi et al. found irregular, thickened corneal nerves with a beaded appearance on white light IVCAM images.[8] The present study showed various patterns of corneal nerve changes in cases of early-stage AK with RK. While some eyes demonstrated thin, tortuous, fragmented nerves in the basal epithelial layer [Fig. 2c], Bowman’s layer [Fig. 2d], and stroma [Fig. 2h] without significant alternations in the morphology or diameter at different corneal depths, some demonstrated thin, fragmented corneal nerves in the basal epithelial layer [Fig. 3a] that became thickened, tortuous, and denser after penetrating the corneal stroma [Fig. 3b-d]. Several bright, irregular, wedge-shaped structures suspected to be Acanthamoeba trophozoites were found attached to the corneal stromal nerves [Fig. 3d-f]. The serial images in Fig. 3 suggest that Acanthamoeba gained access to the corneal stroma by following the corneal epithelial nerves that penetrated Bowman’s layer to enter the stroma, thus causing the nerves to undergo significant morphological changes. To the best of the authors’ knowledge, such a presentation has not been previously reported. There are two main reasons for this observation not having been reported by other studies. First, most of the other studies used HRT instead of Confoscan as the imaging tool[13-20] and the different optical designs may have yielded different results. Because images of the transformed corneal nerves obtained with Confoscan can mimic fungal hyphae [Figs. 2h, i and 3c, d], careful evaluation of the three-dimensional structure and differentiation of deformed corneal nerves from fungal hyphae are necessary. Second, the interpretation of IVCAM images is mostly dependent on the morphological presentation, and it cannot be contested with certainty that the bright linear areas presented in Fig. 3 were corneal nerves and not stromal tracts created during the migration of trophozoites into the stroma and along the corneal nerves. The findings of a study by Kiderlen et al., where a migratory pathway of the “brain-eating” amoeba Naegleria fowleri was found, support this possibility. It was theorized that amoebae can invade the olfactory epithelium by traveling along the olfactory nerves via holes in the cribiform plate and reach the olfactory bulb to cause severe necrotizing meningoencephalitis.[20] Therefore, further studies are required to confirm corneal nerve changes and the migration pathway of Acanthamoeba from the ocular surface into the corneal stroma in cases of AK.

This study has some limitations. First, white light IVCAM with an automatic capture technique was used to evaluate the included patients. The disadvantage of this technique is that it is difficult to maintain the patient’s head position. This may explain why Acanthamoeba cysts or trophozoites were detected in neither the epithelium nor the stroma in cases 3 and 17.
Second, even though this was the largest case series using white light IVCM to evaluate early-stage RK with AK, the sample size may have been small, and further larger studies using the manual operating mode of the white light IVCM device are required to confirm these results.

**Conclusion**

In conclusion, the present findings suggest that white light IVCM provides useful information for the diagnosis of early-stage AK with RK. It can detect the presence of *Acanthamoeba* trophozoites and cysts, the honeycomb pattern of activated keratocytes, highly reflective spindle-shaped structures, and changes in the corneal nerves that are characteristic of this disease. Pathological corneal nerves undergo spatial changes at different depths, and careful interpretation of this finding is important for an accurate diagnosis of AK with RK.

**Declaration of patient consent**

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

**References**

1. Naginton J, Watson PG, Playfair TJ, McGill J, Jones BR, Steele AD. Amoebic infection of the eye. Lancet 1974;2:1537-40.
2. Vaddavalli PK, Garg P, Sharma S, Sangwan VS, Rao GN, Thomas R. Role of confocal microscopy in the diagnosis of fungal and *acanthamoeba* keratitis. Ophthalmolmology 2011;118:29-35.
3. Tu EY, Joslin CE, Sugar J, Booton GC, Shoff ME, Fuerst PA. The relative value of confocal microscopy and superficial corneal scrapings in the diagnosis of *acanthamoeba* keratitis. Cornea 2008;27:764-72.
4. Parmar DN, Awwad ST, Petroll WM, Bowman RW, McCulley JP, Cavanagh HD. Tandem scanning confocal corneal microscopy in the diagnosis of suspected *acanthamoeba* keratitis. Ophthalmology 2006;113:538-47.
5. Kanavi MR, Javadi M, Yazdani S, Mirdehghan S. Sensitivity and specificity of confocal scan in the diagnosis of infectious keratitis. Cornea 2007;26:782-6.
6. Pfister DR, Cameron JD, Krachmer JH, Holland EJ. Confocal microscopy findings of *acanthamoeba* keratitis. Am J Ophthalmol 1996;121:119-28.
7. 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.
8. Szaflik JP. Comparison of *in vivo* confocal microscopy of human cornea by white light scanning slit and laser scanning systems. Cornea 2007;26:436-45.
9. Kobayashi A, Yokogawa H, Yamazaki N, Ishibashi Y, Oikawa Y, Tokoro M, et al. *In vivo* laser confocal microscopy findings of radial keratoneuritis in patients with early stage *acanthamoeba* keratitis. Ophthalmolmology 2013;120:1348-53.
10. Yamazaki N, Kobayashi A, Yokogawa H, Ishibashi Y, Oikawa Y, Tokoro M, et al. *In vivo* imaging of radial keratoneuritis in patients with *acanthamoeba* keratitis by anterior-segment optical coherence tomography. Ophthalmology 2014;121:2153-8.
11. Moore MB, McCulley JP, Kaufman HE, Robin JR. Radial keratoneuritis as a presenting sign in *acanthamoeba* keratitis. Ophthalmolmology 1986;93:1310-5.
12. Alfawaz A. Radial keratoneuritis as a presenting sign in *acanthamoeba* keratitis. Middle East Afr J Ophthalmol 2011;18:252-5.
13. Kobayashi A, Ishibashi Y, Oikawa Y, Yokogawa H, Sugiyama K. *In vivo* and *ex vivo* laser confocal microscopy findings in patients with early-stage *acanthamoeba* keratitis. Cornea 2008;27:439-45.
14. Shiraiishi A, Uno T, Oka N, Hara Y, Yamaguchi M, Ohashi Y. *In vivo* and *in vitro* laser confocal microscopy to diagnose *acanthamoeba* keratitis. Cornea 2010;29:861-5.
15. Rezaei Kanavi M, Naghshgar N, Javadi MA, Sadat Hashemi M. Various confocal scan features of cysts and trophozoites in cases with *acanthamoeba* keratitis. Eur J Ophthalmol 2012;22:S46-50.
16. Tu EY. *Acanthamoeba* and other parasitic corneal infections. In: Kuchler JE, Mannis MJ, Holland EJ, editors. Cornea. St. Louis MO: Mosby; 2011. p. 1023-32.
17. Kurbayan K, Hoesl LM, Schrems WA, Hamrah P. Corneal nerve alterations in acute *acanthamoeba* and fungal keratitis: An *in vivo* confocal microscopy study. Eye (Lond) 2012;26:126-32.
18. Muller RT, Abedi F, Cruzat A, Witkin D, Baniasadi N, Cavalcanti BM, et al. Degeneration and regeneration of subbasal corneal nerves after infectious keratitis: A longitudinal *in vivo* confocal microscopy study. Ophthalmology 2015;122:2200-9.
19. Cruzat A, Schrems WA, Schrems-Hoesl LM, Cavalcanti BM, Baniasadi N, Witkin D, et al. Contralateral clinically unaffacted eyes of patients with unilateral infectious keratitis demonstrate a sympathetic immune response. Invest Ophthalmol Vis Sci 2015;56:6612-20.
20. Hau SC, Dart JK, Vesaluoma M, Parmar DN, Claerhout I, Bibi K, et al. Diagnostic accuracy of microbial keratitis with *in vivo* scanning laser confocal microscopy. Br J Ophthalmol 2010;94:982-7.
21. Matsumoto Y, Dogru M, Sato EA, Katono Y, Uchino Y, Shimmura S, et al. The application of *in vivo* confocal scanning laser microscopy in the management of *acanthamoeba* keratitis. Mol Vis 2007;13:1319-26.
22. Labbe A, Khammari C, Dupas B, Gabison E, Brasnau E, Labetoulle M, et al. Contribution of *in vivo* confocal microscopy to the diagnosis and management of infectious keratitis. Ocul Surf 2009;7:41-52.
23. Kiderlen AF, Laube U. Balamuthia mandrillaris, an opportunistic agent of granulomatous amebic encephalitis, infects the brain via the olfactory nerve pathway. Parasitol Res 2004;94:49-52.