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The Outcome of Concurrent Intracameral Fluconazole Injection with Amniotic Membrane Transplantation for Fungal Keratitis in Dr. M. Djamil General Hospital: A Case Series

Ferry Indratno^{1*}, Havriza Vitresia¹

¹Department of Ophthalmology, Faculty of Medicine, Universitas Andalas/Dr. M. Djamil General Hospital, Padang, Indonesia

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*Corresponding author:

Ferry Indratno

E-mail address:

ferry.indratno1@gmail.com

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ABSTRACT

Background: Fungal keratitis is often difficult to eradicate and requires long treatment. Antifungal agents have lower efficacy because of low tissue penetration and the indolent nature of the infection. Fluconazole has effective ocular penetration ability, reaching aqueous concentration as same as plasm concentration. Multilayer amniotic membrane transplantation (MLAMT) has been shown to be a promising surgical procedure to reconstruct the ocular surface by supporting the epithelialization of the cornea and having protective antimicrobial properties. This study aims to report the outcome of concurrent intracameral fluconazole injection with amniotic membrane transplantation for fungal keratitis in Dr. M. Djamil General Hospital. **Case presentation:** Four inpatients in the ophthalmology ward of Dr. M. Djamil General Hospital with moderate to severe fungal keratitis. All patients were first treated with medications. Because the patients did not show clinical improvement after getting maximum therapy, they underwent intracameral fluconazole injection concurrent with MLAMT. MLAMT was performed with multilayered amniotic membranes to fill and cover the defect. The bandage contact lens was placed to protect and keep the MLAMT in place and for patients' comfort. Patients were then observed for signs of inflammatory reaction and epithelialization. All patients showed reduced inflammation and improved epithelialization after surgery. **Conclusion:** Concurrent intracameral fluconazole injection with MLAMT can be considered an alternative treatment for refractory fungal keratitis as it decreases the rate of inflammation and improves epithelialization.

1. Introduction

Fungal keratitis is less common than bacterial keratitis, accounting for less than 10% of corneal infections.¹ It is more common in the developing countries as compared to the developed countries.² Problems encountered in cases of fungal keratitis include establishing the correct clinical diagnosis and obtaining confirmation of the fungal organisms on laboratory diagnosis.³ Problems related to therapy include suboptimal penetration of the antifungal drugs, difficulty in preparation, and availability of the antifungal medications.^{3,4}

Antifungal agents for fungal keratitis can be given as topical/systemic agents orally or local injections like in targeted therapy with intra-cameral and intracorneal injections. The following classes of drugs are used as therapy: polyenes (amphotericin B, natamycin, nystatin), azoles/imidazoles (ketoconazole, miconazole, econazole), triazoles (itraconazole, voriconazole, posaconazole, fluconazole, ravuconazole) and echinocandins (caspofungin, micafungin, anidulafungin).^{5,6}

Targeted drug delivery can be in the form of intrastromal and intracameral injections of antifungal

agents. Targeted drug delivery has an advantage by avoiding sub-optimal therapeutic levels and giving better penetration of antifungal agents, especially in keratitis with deep stromal involvement. Fluconazole is a synthetic bis-triazole available in oral, topical, and intravenous preparations. It is shown to have a low side effect profile and good intraocular penetration.^{7,8}

Fluconazole has the ability to penetrate the eye, which is quite effective, and can reach aqueous concentrations equal to plasma concentrations. Oral use of 150-300 mg daily is effective for ocular therapy. Intracameral injection of fluconazole is administered directly into the anterior ocular chamber for eradication of fungal hyphae/yeast without the need for modification by corneal esterase. The concentration achieved is also higher than externally administered. The advantage of intracameral injection of fluconazole is that it does not undergo a corneal esterase reaction, and the concentration is accurate directly in the anterior chamber.⁷

The amniotic membrane, or amnion, is the innermost layer of the placenta and consists of a thick basement membrane and an avascular stromal matrix.⁹ Transplantation of amniotic membranes has been shown to be a promising surgical procedure that is helpful in reconstructing the ocular surface by supporting the epithelialization of the cornea. They also have protective antimicrobial properties, anti-inflammatory and antiangiogenic properties. This study aims to report the outcome of concurrent intracameral fluconazole injection with amniotic membrane transplantation for fungal keratitis in Dr. M. Djamil General Hospital.

2. Case Presentation

Four inpatients in the ophthalmology ward of Dr. M. Djamil General Hospital with moderate to severe fungal keratitis were evaluated in this study. All patients were first treated with medications. After no clinical improvement, patients were planned for surgery. Paracentesis of the hypopyon and then intracameral fluconazole injection were done. Fluconazole was taken from infusion preparation. It

was taken with a dose of 0.025 mg in 0.1 ml. The MLAMT was then shaped and placed in 2-4 layers to fill the ulcers and corneal perforation. A bandage contact lens was placed on the cornea to maintain the MLAMT in place and facilitate epithelialization. The explanation for each case is described below.

Case 1

Mrs. M, 40 years old, came to Dr. M. Djamil Hospital with a chief complaint of blurred and red eye in the right eye a week before. The right eye was stuck by twigs then she dripped herb water into her eyes. Visual acuity (VA) of the right eye (RE) was 1/300, with palpebral edema, conjunctival and ciliary injections, central corneal ulcer with a diameter 4 mm, 1/3 stromal depth. Unfortunately, microbiology laboratory examination failed to find hyphae. The patient was clinically diagnosed with fungal keratitis in the right eye. The patient was treated with fluconazole eye drop (ED) 1 drop/4 hours, levofloxacin ED 1 drop/4 hours, sulfas atropine ED 1 drop/8 hours, EDTA ED 1 drop/6 hours and fluconazole oral 150 mg/day. After there were no clinical improvements for a few days, the patient was then subjected to intracameral fluconazole injection and MLAMT. On follow-up, there was decreased inflammation observed on the third day after surgery. Complete epithelialization was observed on day 15 after surgery (Figure 1).

Case 2

Mr. K, 42 years old, was clinically diagnosed with fungal keratitis in the right eye with hypopyon. VA of RE was 20/200 with palpebral edema, conjunctival and ciliary injection. On the cornea, there was a central corneal ulcer with a size 2x2 mm and 1/3 stromal depth. In the anterior chamber, there was a hypopyon 1 mm in height. The patient was then given medication. After there was no clinical improvement in the ulcer, the patient was then subjected to paracentesis of hypopyon, intracameral fluconazole injection, and MLAMT. On follow-up, there was decreased inflammation observed on day 3 after surgery. Complete epithelialization was observed on

day 15 after surgery (Figure 2).

Case 3

Mr. M. 54 years old, was clinically diagnosed with fungal keratitis in the right eye with hypopyon. Patient with a history of dripping herb water into his right eye because of feeling discomfort. VA of RE was 1/~ good projection. There was palpebral edema, conjunctival and ciliary injections, and a central corneal ulcer with a diameter 6 mm, 1/3 stromal depth, and endothelial plaque positive. Despite the maximum therapy given, the patient did not show clinical improvement. The patient then underwent paracentesis of hypopyon, intracameral fluconazole injection, and MLAMT. On follow-up, decreased inflammation was observed on day 5 after surgery. Complete epithelialization was observed at day 16 after surgery (Figure 3).

Case 4

Mr. K, 62 years old, was clinically diagnosed with central fungal keratitis on the right eye with hypopyon. Patient with history of washing his right eye with tap water. VA of RE was 1/~ and good projection. There was palpebral edema, conjunctival and ciliary injections, and a central corneal ulcer with a diameter 7 mm, 2/3 stromal depth, and endothelial plaque. Despite the maximum therapy given, the patient did not show clinical improvement, and the patient was then subjected to paracentesis of hypopyon, intracameral fluconazole injection, and MLAMT. In this case, there was decreased inflammation and reduction of endothelial plaque at 5 days after surgery. In the second week of follow-up, there was no longer any endothelial plaque, and the ulcer was completely epithelialized (Figure 4).



Figure 1. A. Case 1 preoperative B. Post MLAMT C. Day 14 after surgery.

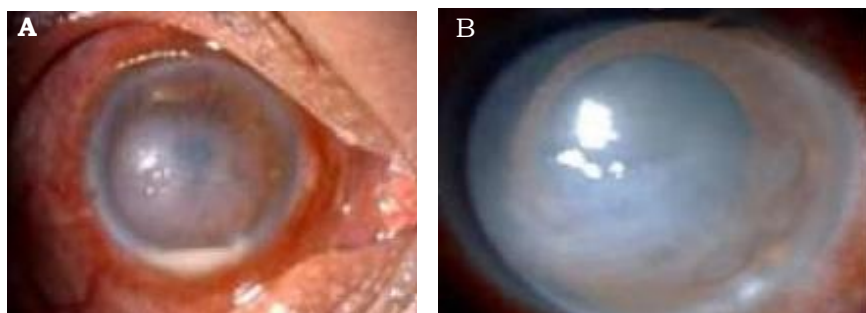


Figure 2. A. Case 2 preoperative. B. Day 15 after surgery, VA 20/150.

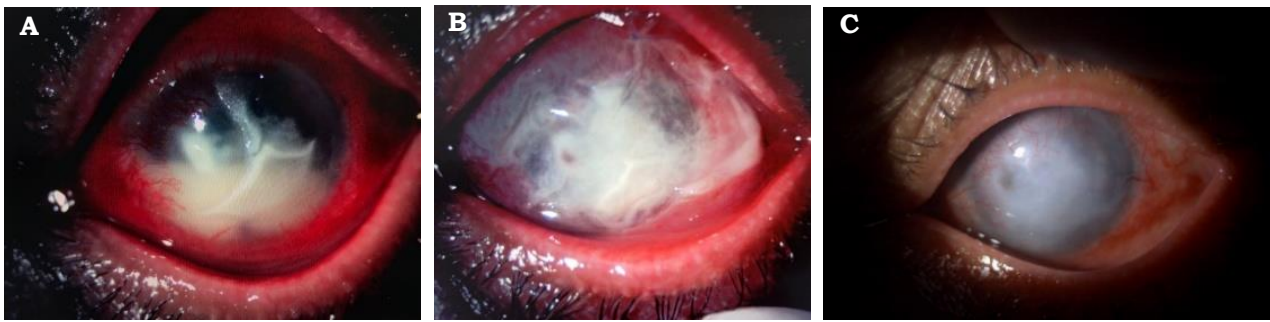


Figure 3. A. Case 3 preoperative B. Post MLAMT C. Day 14 after surgery.

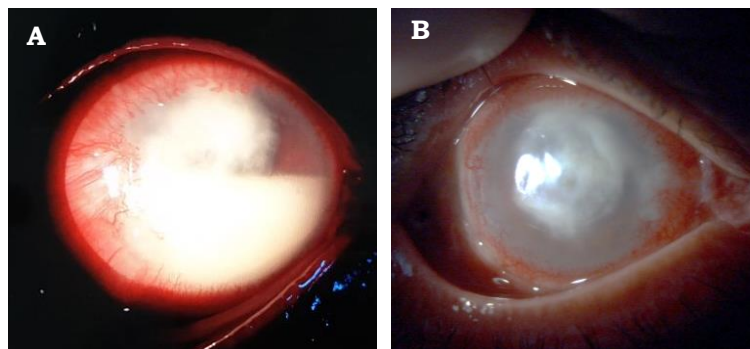


Figure 4. A. Case 4 preoperative B. Days 14 after surgery.

3. Discussion

Fungal keratitis is the etiology of visual loss in tropical and developing countries. In some hot and humid countries, it accounts for 50% of cases.¹⁰ Risk factors for infection are trauma (65% of cases in tropical areas), especially trauma with vegetation, epithelial defects, diabetes, systemic immunosuppression, and contact lenses. In the USA, the use of contact lenses is a risk factor for as much as 37% compared to 25% of trauma cases. The opposite occurs in developing countries such as India and Thailand, fungal keratitis is more often related to trauma, and cases caused by contact lenses are rare. In these countries, fungal keratitis accounts for 40% of cases of bacterial keratitis. In India, the estimated incidence of fungal keratitis is 113 per 100,000, with *Aspergillus* being the main etiology. If the infection involves the anterior chamber, management becomes

more difficult for fungal eradication. Yeast-type fungal keratitis is most often caused by *Candida*. The initial clinical signs of fungal infection are quite typical, manifested in the form of lesions or ulcers with a raised surface, grayish-white infiltrates with a dry and rough texture with feathery edges, satellite lesions, and hypopyon with a convex surface. Intraocular expansion of the cornea can occur.¹¹ In these four cases, there were typical clinical manifestations of fungus, such as endothelial plaque, infiltration with feathery edges, dry and rough texture, and hypopyon with a bulging surface.

Patients with fungal keratitis in the early period tend to have fewer inflammatory signs and symptoms than bacterial keratitis and may have fewer injections. Filamentous fungal keratitis manifests as a grayish-white, dry infiltrate with irregular edges and feathery or filamentous. Superficial lesions appear grey-white,

raise the surface of the cornea, and have a rough, dry, gritty texture. Occasionally, satellite or multifocal infiltrates may be seen, although these have rarely been reported. Deep stromal infiltrates may occur in the intact epithelium. Endothelial plaque and/or hypopyon may also occur if the fungal keratitis is deep or extensive.

Antifungal therapy is divided into the following 3 groups; polyenes, azoles, and pyrimidines. Fluconazole is a first-generation antifungal, which differs from previous azole antifungals by its structure containing a triazole ring. Imidazole antifungals are primarily used topically. Fluconazole and certain triazoles are preferred when systemic therapy is required because of their safety and good absorption when given orally. Fluconazole is effective against most yeasts, for example, *Candida* (but not *Candida krusei* or *Candida glabrata*), *Cryptococcus neoformans*, some dimorphic fungi, and dermatophytes. Fluconazole does not have a significant effect on mold/filamentose fungi, such as *Aspergillus* and *Fusarium*. Unlike other azole groups, fluconazole is water soluble.^{12,13}

Fluconazole has a primary function as a fungistatic agent but can act as a fungicide for certain organisms in a dose-dependent manner, especially *Cryptococcus*. After oral administration, fluconazole is absorbed almost completely within two hours. Fluconazole eye drops can reach good therapeutic levels with intracorneal injection, but with intracameral injection, the drug is received directly into the anterior chamber without the need to be modified by corneal esterase, the concentration achieved is also higher than given externally (eye drops). Each layer of the cornea has a different polarity and structure that limits drug permeability. The intracameral injection also has disadvantages, including toxicity that can occur at inappropriate concentrations, pH, and osmolarity, then also infection contamination, and impaired incompatibility in multiple drug combinations.¹³

Esterase is the most important enzyme for the bioconversion of prodrugs in the eye. Esterase activity was reported to be highest in the iris-ciliary body, followed by the cornea and aqueous humor. Drugs

that pass through the cornea after topical administration will pass through the epithelial membrane, which tends to be lipophilic with tight junctions. Prodrugs attached to esterases for bioversion are effective in increasing drug penetration.^{7,8}

The intracameral route of drug administration is gradually evolving into a new modality for the management of keratitis, especially those caused by fungi. In severe cases, hyphae may penetrate Descemet's membrane intact and colonize the anterior chamber of the eye. Hypopyon usually contains a fungal element, which is difficult to treat because most topical antifungal drugs have poor corneal penetration. Intracameral injection of the drug does not go through a corneal esterase reaction and accurate concentration directly in the anterior chamber, thus a consideration in refractory cases.¹⁴

All patients showed clinical improvements, reduced inflammation, and showed complete epithelization within 2 weeks post-surgery. MLAMT gives anti-inflammatory and anti-scarring effects and contains growth factors that promote epithelial wound healing on the surface of the eye. To further support epithelial healing in cases refractory to local medical therapy, we use bandage contact lenses. An amniotic membrane consists of a thick basement membrane and an avascular stroma that contains a high concentration of basic fibroblast growth factors. It also represents a mechanical barrier for frictional forces. Furthermore, amniotic membranes promote epithelial recovery and suppress inflammation because of the contained cytokines and growth factors. In addition, MLAMT also has protective antimicrobial properties.^{9,15}

4. Conclusion

Concurrent intracameral fluconazole injection with MLAMT can be considered an alternative treatment for refractory fungal keratitis as it decreases the rate of inflammation and improves epithelization.

5. References

1. Acharya Y, Acharya B, Karki P. Fungal keratitis: Study of increasing trend and

- common determinants. *Nepal J Epidemiol.* 2017; 7: 685–93.
2. Kowalski RP, Nayyar SV, Romanowski EG, Shanks RM, Mammen A, Dhaliwal DK, et al. The prevalence of bacteria, fungi, viruses, and acanthamoeba from 3004 cases of keratitis, endophthalmitis, and conjunctivitis. *Eye Contact Lens.* 2020; 46: 265–8.
 3. Mahmoudi S, Masoomi A, Ahmadikia K, Tabatabaei SA, Soleimani M, Rezaie S, et al. Fungal keratitis: An overview of clinical and laboratory aspects. *Mycoses.* 2018; 61: 916–30.
 4. Manikandan P, Abdel-Hadi A, Randhir BSY, Banawas S, Dukhyil AAB, Alshehri B, et al. Fungal keratitis: epidemiology, rapid detection, and antifungal susceptibilities of *Fusarium* and *Aspergillus* isolates from corneal scrapings. *Biomed. Res. Int.* 2019, 2019: 6395840.
 5. Bourcier T, Sauer A, Dory A, Denis J, Sabou, M. Fungal keratitis. *J Fr Ophtalmol.* 2017; 40; e307–e313.
 6. Austin A, Lietman T, Rose-Nussbaumer J. Update on the management of infectious keratitis. *Ophthalmology.* 2017; 124: 1678–89.
 7. Raj N, Vanathi M, Ahmed NH, Gupta N, Lomi N, Tandon R. Recent perspectives in the management of fungal keratitis. *Journal of Fungi.* 2021; 7(11): 907.
 8. Ansari Z, Miller D, Galor A. Current thoughts in fungal keratitis: Diagnosis and treatment. *Curr Fungal Infect Rep.* 2013; 7(3): 209–18.
 9. Tseng SCG. Amniotic membrane transplantation for ocular surface reconstruction. *Biosci Rep.* 2001; 21(4): 481–9.
 10. Inoue Y, Ohashi Y, Suzuki T, Shimomura Y, Fukuda M, Sotozono C, et al. Multicenter prospective observational study of fungal keratitis—current status of patients’ background, clinical findings, treatment and prognosis. *Nippon Ganka Gakkai Zasshi.* 2016; 120: 5–16.
 11. Mahdy RA, Nada WM, Wageh MM, Kader MA, Saleh MM, Alswad MM. Assessment safety and efficacy of a combination therapy of topical amphotericin B and subconjunctival fluconazole for the treatment of fungal keratitis. *Cutan Ocul Toxicol.* 2010; 29(3): 193–7.
 12. Isipradit S. Efficacy of fluconazole subconjunctival injection as adjunctive therapy for severe recalcitrant fungal corneal ulcer. *J Med Assoc Thai.* 2008; 91(3): 309–15.
 13. Prajna NV, Krishnan T, Mascarenhas J, Rajaraman R, Prajna L, Srinivasan M, et al. The mycotic ulcer treatment trial: A randomized trial comparing natamycin vs voriconazole. *JAMA Ophthalmol.* 2013; 131: 422–9.
 14. Ho JW, Fernandez MM, Rebong RA, Carlson AN, Kim T, Afshari NA. Microbiological profiles of fungal keratitis: A 10-year study at a tertiary referral center. *J. Ophthalmic Inflamm. Infect.* 2016; 6: 5.
 15. Casalita V, Nora RLD, Edwar L, Susiyanti M, Sitompul R. Amniotic membrane transplantation for infectious corneal ulcer treatment: a cohort retrospective study. *Medical Journal of Indonesia.* 2020; 29(4): 379–85.