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Case Report

Intrastromal antifungal injection as a successful modality of treatment for fungal keratitis: a case series

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ABSTRACT

Fungal keratitis is a dreaded occurrence in the cornea and anterior segment given the difficulty in treating the disease. Hereby reporting 5 cases of fungal keratitis causes by virulent organisms such as *Aspergillus versicolor*, *Aspergillus fumigatus*, *Phialophora sp*, and *Fusarium sp*. A retrospective interventional case series of 5 fungal keratitis which were successfully treated by intrastromal antifungal injection performed by a single surgeon from March 2017 till April 2018. The fungal keratitis stromal abscess sizes range from largest of 3mm x 2mm to smallest of 1mmx 1mm, mostly located paracentral and one case was noted to have hypopyon measuring about 1mm. On the first week of treatment, patients noted to exhibit poor response to topical antifungal. Hence, intrastromal amphotericin B injection 5mcg/0.1ml about 0.1ml administered into the affected eye ranging from once to 3 times in all patients except for one patient; who is post-operative 1 year penetrating keratoplasty infected with *Phialophora keratitis* is given intrastromal amphotericin B injection 5mcg/0.1ml about 0.1ml for 3 times and intrastromal voriconazole injection 50mcg/0.1ml about 0.1ml for 3 times. Within next four weeks, all the 5 cases of fungal keratitis became completely quiet with healed epithelial defect and corneal scarring. In summary, the intrastromal antifungal injection can constitute a good modality for the treatment of recalcitrant cases of fungal keratitis, revealing highly potent antifungal effects as the medication is administrated directly to the site of keratitis, promises shorter recovery period, and early intrastromal antifungal injection also leads to quicker healing with good vision prognosis.

Keywords: Amphothericin B, Fungal keratitis, Intrastromal injection, Penetrating keratoplasty

INTRODUCTION

Fungal keratitis is a dreaded occurrence in the cornea and anterior segment given the difficulty in treating the infection, starting from the availability of and susceptibility to antifungal agents, to penetration of corneal tissues, as fungal pathogens tend to deeply penetrate corneal tissues and may reach the anterior chamber. The keratitis may worsen and lead to serious complications such as corneal descemetocele, staphyloma, endophthalmitis, melting, perforation, and blindness. The most frequently isolated organisms of fungal keratitis are *Aspergillus sp.* and *Fusarium sp.*, which are rather exceptionally virulent microorganisms and are partially resistant to most of the antifungal medications.[1] The hypha is adept of penetrating the intact Descemet's membrane and rapidly invade the anterior chamber. In such cases, conventional antifungal agents such as fluconazole, topical natamycin, amphotericin B, or the combination with oral fluconazole, seems to yield poor outcome. Furthermore, the corneal penetration and bioavailability of many of the conventional topical antifungal preparations are suboptimal due to the large molecular size of antifungal agents, making it challenging to treat cases of recalcitrant fungal keratitis.[2,3] Therapeutic keratoplasty may be an effective way to eradicate the fungal infection, however it is not as effective when performed in a quiescent healed eyed.[4,5] In

order to improvise the treatment of fungal keratitis, researchers have evaluated alternate routes such as intracameral or intrastromal amphotericin B injections to treat fungal keratitis.[6-8] Amphotericin B as a topical antifungal agent, has broad-spectrum antifungal activity but it has poor corneal penetration due to its large molecular size and impose strong cytotoxicity at higher concentrations.[9,10] In a study conducted on rabbit eyes, the intracameral antifungal injection is able to achieve effective drug concentration in aqueous humor rapidly, however in intracameral mode drug level in aqueous humor drops abruptly within one day whereas in intrastromal mode, after a single injection, effective drug level is maintained longer for about 7 days.[9]

Hence, in this study, authors have used intrastromal antifungal injections of amphotericin B and voriconazole as an alternative to conventional therapies and evaluated its efficacy in the management of keratomycosis which was resistant to conventional antifungal medical treatment and may have requisite potential surgical intervention if deteriorated further.

CASE REPORTS

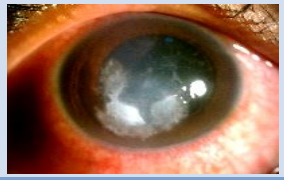
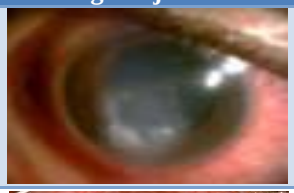
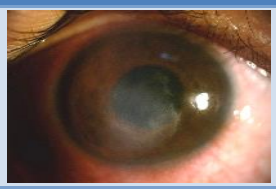
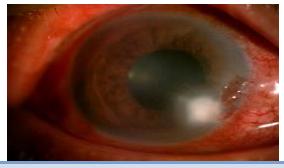
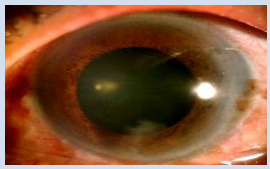
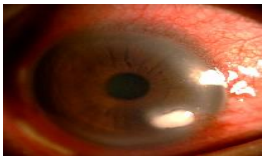
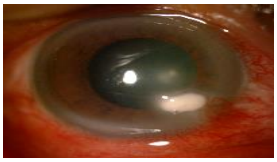
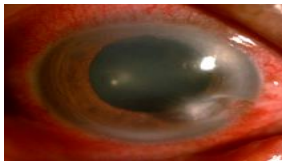
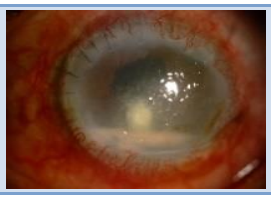
This is a retrospective interventional case series. Case records of 5 patients from March 2017 till April 2018 performed by a single surgeon were reviewed retrospectively. The epidemiological characteristics, presenting history, clinical features, corneal scrapping fungal identification results, treatment protocol, and final outcome data for each patient were reviewed (Table 1).

Table 1: Clinical data of patients with fungal keratitis treated with intrastromal antifungal injection.

Case no.	Age/Sex/ Ethnicity/ Occupation/ Co-morbidties	History	Duration of onset of symptoms (days)	Initial BCVA	Size of ulcer (mm)	Fungal identification	Total no. of intrastromal injection	Final BCVA	Duration of treatment (days)	Healed with
1	60/Male/ Malay/ Gardener/ HPT	Foreign body (gardening)	14	6/36	3.2x2.4	*Fusarium sp.*	2	6/9	29	Scarring
2	47/Male/ Indonesian/Palm oil worker/ NKMI	Palm oil debris	10	6/9	3.0x1.0	*Aspergillus versicolor*	3	6/9	38	Scarring
3	60/Female/ Indian/Rubber tapper/ NKMI	Rubber plant particles	6	6/12	2.0x2.0	*Fusarium sp.*	2	6/6	20	Scarring
4	54/Male/ Malay/ Forest Officer/ HPT	Foreign body	7	6/9	3.0x1.0	*Aspergillus fumigatus*	2	6/6	28	Scarring
5	43/Male/ Malay/ Rubber tapper/ NKMI/Post-operative 1 year LE therapeutic PK for pseudomonas corneal ulcer	Corneal trauma	4	HM	2.2x1.8	*Phialophora sp.*	6	1/60	35	Scarring

All cases of fungal keratitis which were already on gutt fortified gentamicin 0.9% and Gutt fortified ceftazidime 5% every 15 minutes for first 2 hours then every half hourly, gutt amphothericin B 0.15% and gutt fluconazole 0.2% every half hourly together with tab fluconazole 100mg BD, however noted to worsen within first week of treatment. Thereafter, treated successfully with intrastromal antifungal injection either intrastromal amphotericin B injection 5µg/0.1ml about 0.1ml or intrastromal voriconazole injection 50µg/0.1ml about 0.1ml were included.[11,12]

Table 2: Anterior segment photos (16x) of patients on presentation, before intrastromal antifungal injection, after intrastromal antifungal injection and upon discharge.

Case	On presentation	Before intrastromal antifungal injection	After intrastromal antifungal injection	Upon discharge
1				
2				
3				
4				
5				

Intrastromal injection was given after taken written consent from patients.

During procedure, injection was bevel down with 26-gauge needle inserted obliquely from the uninvolved, clear area of cornea to reach at the mid-stromal level; in 4-6 divided doses around the ulcer to form a drug deposit or hydration around the circumference of the lesion. The total amount of drug injected intrastromally about 0.1mL each time. Daily anterior segment photos of the fungal keratitis were taken using standardized 16x magnification throughout treatment (Table 2).

Case 1

A 60 years old Malay gentleman with underlying hypertension presented two weeks after alleged hit by foreign body over right eye while lawn moving and sustained right eye pain and blurring of vision. His best corrected vision over right eye was 6/36. The right eye conjunctiva was injected with paracentral corneal ulcer measuring 3.2mmx 2.4mm from 5 to 9 o'clock, with surrounding multiple stromal infiltration. AC cells were 3+ with no hypopyon. The patient was admitted and started with loading dose of gutt fortified gentamicin 0.9% and gutt fortified ceftazidime 5% every 15 minutes for first 2 hours then every half hourly, gutt amphothericin B 0.15% and gutt fluconazole 0.2% every half hourly together with tab fluconazole 100mg BD. Patient exhibited poor response till day 5 of treatment with increasing infiltration and stromal abscess thickness. The right eye corneal scrapping culture and sensitivity was noted to be *Fusarium sp*. Intrastromal amphothericin B injection was given twice on day 5 and 7 of treatment, subsequently patient exhibited good response with reducing ulcer size and subsequently resolution of the infection. The corneal ulcer healed with paracentral scarring at day 29 of treatment with best corrected vision of 6/9.

Case 2

A 47 years old Indonesian gentleman who is a palm oil worker with no known medical illness presented with alleged hit by palm oil debris over right eye while working in palm oil estate 10 days ago. There was history

of single agent topical antibiotic given at general practitioner for first one week, however his right eye pain and redness worsened in next 10days. His best correct vision over right eye was VA 6/9. The left eye conjunctiva was injected with paracentral stromal abscess measuring 3mmx 1mm at 4 o'clock, AC cells were 4+, with no hypopyon. The patient was admitted and started with loading dose of gutt fortified gentamicin 0.9% and gutt fortified ceftazidime 5% every 15minutes for first 2 hours then every half hourly, gutt amphothericin B 0.15% and gutt fluconazole 0.2% every half hourly together with tab fluconazole 100mg BD. The stromal abscess size increased daily daily and measured 3mmx 2.4mm at day 4 of treatment with dropping in vision to 6/19. Patient was given intrastromal amphothericin B three times on day 4, 7 and 11 of treatment, subsequently stromal abscess resolved gradually. The right eye corneal scrapping culture and sensitivity result was *Aspergillus versicolor*. The corneal ulcer healed with scarring at day 38 of treatment with vision 6/9.

Case 3

A 60 years old Indian lady who works as a rubber tapper with no known medical illness presented with alleged hit by rubber plant particles over left eye while rubber tapping in estate, sustained left eye pain and redness for 6 days prior to presentation at eye clinic. Her best corrected left eye vision was 6/12. Her left eye conjunctiva was injected with paracentral corneal ulcer measuring 2mmx 2mm at 5 o'clock, AC cells were 3+with no hypopyon. The patient was admitted and started with loading dose of gutt fortified gentamicin 0.9% and gutt fortified ceftazidime 5% every 15 minutes for first 2 hours then every half hourly, gutt amphothericin B 0.15% and gutt fluconazole 0.2% every half hourly together with tab fluconazole 100mg BD. The corneal ulcer size was static till day 3 of treatment. Hence, intrastromal amphothericin B injection was two times on day 3 and 5 of treatment, subsequently patient exhibited rapid recovery. The left eye corneal scrapping culture and sensitivity result was *Fusarium sp*. The left eye corneal ulcer healed completely with paracentral scarring at day 20 of treatment with vision 6/6.

Case 4

A 54 years old Malay gentleman who works as forest officer with underlying hypertension complained of foreign body entered right eye while working in forest, sustained right eye pain and redness for past one week. His best corrected vision over right eye was 6/9. The right eye conjunctiva was injected with paracentral stromal abscess measuring 3mmx 1mm at 3 o'clock, AC cells were 3+ with no hypopyon. The patient was admitted and started with loading dose of gutt fortified gentamicin 0.9% and gutt fortified ceftazidime 5% every 15 minutes for first 2 hours then every half hourly, gutt amphothericin B 0.15% and gutt fluconazole 0.2% every half hourly together with tab fluconazole 100mg BD. The corneal ulcer size was static till day 5 of treatment. Patient was given intrastromal amphothericin B injection twice on day 5 and 7 of treatment, subsequently showed drastic improvement with complete resolution of stromal abscess. The right eye corneal scrapping culture and sensitivity result was *Aspergillus fumigatus*. The right eye corneal ulcer healed with scarring at day 28 of treatment with vision 6/6.

Case 5

A 43 years old Malay gentleman who is a rubber tapper with post-operative 1 year left eye therapeutic penetrating keratoplasty for pseudomonas corneal ulcer with early graft failure presented with alleged hit by daughter's hand over left eye, then sustained pain and redness for 4 days. On arrival, his left eye best corrected vision was hand movement. Patient's left eye conjunctiva was injected with paracentral corneal ulcer measuring 2.2mmx 1.8mm at 6 o'clock, AC cells were 3+ and there was presence of hypopyon measuring 1mm. The patient was admitted and started with loading dose of gutt fortified gentamicin 0.9% and gutt fortified ceftazidime 5% every 15 minutes for first 2 hours then every half hourly, gutt amphothericin B 0.15% and gutt fluconazole 0.2% every half hourly together with tab fluconazole 100mg BD. The corneal ulcer worsened rapidly at day 3 of treatment with increasing hypopyon measuring 1.8mm at day 4 of treatment. Intrastromal amphotericin B injection was given for 3 times at day 4, 6, and 8 of treatment initially. However, patient exhibited slow response to treatment. Patient was counselled for therapeutic penetrating keratoplasty at this point, however patient refused for another penetrating keratoplasty and requested for non-surgical intervention. The right eye corneal scrapping culture and sensitivity result was noted to be *Phialophora sp*. Hence, intrastromal voriconazole injection was given for 3 times at day 10, 12 and 15 of treatment to patient. The corneal ulcer started improving. Eventually, the left eye corneal ulcer healed with scarring at day 35 of treatment with vision 1/60.

DISCUSSION

Management of fungal keratitis is challenging in view of fungistatic effect of most of the topical antifungal agents and their poor penetration to the deeper layers of the cornea leading to suboptimal therapeutic levels at the site of infection. Topical antifungal alone or combined with oral antifungal medications seems to be effective in the early stages of the keratitis. Therapeutic keratoplasty may be an effective way to control the fungal infection. However, keratoplasty is not as effective as when performed on a quiescent eye after healing and partly because of the limited and erratic supply of donor cornea.

Targeted drug delivery has the potential to achieve sufficient drug concentrations at the site of infection and serve as an alternative modality of treatment in eyes with recalcitrant fungal keratitis. Among the targeted drug

delivery modalities, intrastromal injection of antifungal agents has shown promising results. In order to achieve adequate intracorneal concentrations of antifungals, intrastromal injections of antifungals have been tried.[6] This mode treatment was used in infections which were generally focused in the cornea and seldom invaded the anterior chamber.

Amphotericin B has been proven to be an effective drug in treating systemic mycosis caused by natamycin-resistant filamentous fungi.[13] It has a broad spectrum of activity but has certain degree of cytotoxicity and poor penetration.[9,10] It remains to be a potent agent in the treatment of fungal keratitis, and its efficacy is reliant on the ability to achieve optimal drug levels in the cornea.[3,13] Therefore, to enhance the efficacy of amphotericin B, choosing a proper formulation and mode of application is the key.[7] The ideal dose of amphotericin B for intrastromal use is undetermined, but it should achieve maximum therapeutic effect with minimal side effects. Study has concluded that intrastromal injection of amphotericin B at a concentration of less than 10µg per 0.1mL is non-toxic in rabbit corneas, and at a concentration of 5µg per 0.1mL it does not appear to be harmful to keratocytes or endothelial cells in the clinic.[6,14] As per in present case series, we have used intrastromal amphotericin B at concentration of 5µg /0.1ml as most of the keratitis were focused on the cornea.

Sharma et al, reported that there is requirement of two or more intrastromal antifungal injections to attain optimal response.[15] Similarly, in our study all the 5 cases initially showed poor response to topical antifungal treatment, however after given intrastromal antifungal injection for two to three times; the recovery was faster and also assured better vision prognosis. Besides that, no toxic effects of intrastromal injection of amphotericin B or voriconazole, such as persistent corneal haze, oedema, corneal erosion, and corneal melting were noted. The topical eye drops were tapered down as the corneal ulcer improved. Furthermore, within next four weeks, all the 5 cases of fungal keratitis became completely quiet with healed epithelial defect and corneal scarring. There was no recurrence of the infection upon withdrawal of all of the antifungal agents.

Aspergillus keratitis and *Fusarium keratitis* in present series were very responsive to intrastromal amphotericin B as per contrast to one case of *Phialophora keratitis* which exhibited resistance to amphotericin B. There are not many cases of *Phialophora keratitis* have been reported since the first and second cases were reported by Wilson et al, and by Polack et al, respectively.[16,17] Wilson et al, showed resistance of Phialophora to amphotericin B, while Polack et al, showed that it was resistant to natamycin.[16,17] In view the high resistance to antifungal agents prior to the discovery of triazoles, there is a variety of surgical treatments being applied for *Phialophora keratitis*, from conjunctival flap, corneal transplantation, to evisceration or enucleation.[18] Recently Li Y et al, concluded that the newer triazoles such as voriconazole had low MICs for Phialophora but that amphotericin B and fluconazole had relatively high MICs/MECs in vitro.[19] Therefore, in case-5 with *Phialophora keratitis* in post penetrating keratoplasty eye when exhibited resistance to amphotericin B authors attempted intrastromal voriconazole injection. This is mainly because patient opted for non-surgical intervention and refused for another penetrating keratoplasy. After given 3 doses of intrastromal voriconazole injection at the concentration of 50µg/0.1ml, patient recovered completely with scarring.[12]

CONCLUSION

In summary, the intrastromal antifungal injection can constitute a good modality for the treatment of recalcitrant cases of fungal keratitis, revealing highly potent antifungal effects as the medication is administrated directly to the site of keratitis, promises shorter recovery period, and early intrastromal antifungal injection also leads to quicker healing with good vision prognosis.

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