

Non-traditional Route for Three Antifungal Drugs in Fungal Keratitis Treatment

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Authors' contributions

This work was carried out in collaboration among all authors. Author MSA designed the study. Author CRS collected the data, wrote the protocol and managed the literature searches. Author MSH performed the statistical analysis and wrote the first draft of the manuscript. All authors read, revised and approved the final manuscript.

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ABSTRACT

Aims: This study aims to evaluate the efficacy of the intrastromal route of antifungal drugs in treatment of fungal keratitis.

Study Design: Prospective non-controlled (single arm) interventional clinical study
Place and Duration of Study: Ophthalmology Department, Assiut University Hospital, between March 2016 to November 2017.

Methodology: Corneal scrapings for direct smears and cultures were done for eighteen eyes of 18 consecutive patients and intrastromal injections were given according to sensitivity results.

Results: After one month of follow up of the cases in this study, 33.3% of the cases had rapid complete cure and the rest had incomplete cure with resolution of ulcer sizes by 55.34% \pm 6.78 after one or more intrastromal injection of antifungal drugs.

Conclusion: Intrastromal injection of antifungal drugs in treatment of fungal keratitis shows promising results in advanced cases of fungal keratitis when combined with the topical route.

Keywords: Fungal keratitis; intrastromal injection; antifungal drugs; fluconazole; amphotericin B; voriconazole.

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1. INTRODUCTION

Fungal keratitis is a devastating ocular infection leading to significant visual morbidity caused by a variety of fungal species capable of colonizing ocular tissues. [1]

Trauma to the corneal epithelium (by means of e.g. vegetative matter, previous eye surgery or contact lens abuse) leads to an epithelial defect through which fungi can gain access to the corneal stroma and cause inflammation and necrosis. Consequently, the organisms can cause intraocular infection when they reach the anterior chamber and posterior segment through penetrating an intact Descemet's membrane. Further damage can be caused by mycotoxins and proteolytic enzymes. [2] Previous literature described the occurrence of fungal keratitis secondary to fungal endophthalmitis. [3]

It was found that filamentous fungi are predominant in hot climates, commonly preceded by previous agricultural trauma, while yeasts are more frequent in temperate ones. [4] Risk factors, other than geographic ones, include ocular surface disease, topical steroid use and immunocompromised patients. [5,6]

Fungal keratitis is primarily a clinical diagnosis based on highly suggestive clinical picture including; feathery borders, raised surface, satellite lesions, presence of a gutter surrounding a white "immune ring" and a thick, cheesy and dome-shaped hypopyon. [7] Meanwhile, some cases may be confusing, and a laboratory workup is mandatory for organism specification and drug choice based on sensitivity results. Tools for laboratory diagnosis include corneal scraping for direct smear examination with special stains under microscope and specific culture media such as Sabouraud dextrose agar. Other methods include ELISA, electrophoresis and immune diffusion; all of which depend on identifying enzymes produced by the fungus. Confocal microscopy aids in fungus identification. [8]

Treating fungal keratitis is basically through medical route, where antifungal preparations are used in the form of eye drops. The most common and commercially available one is the polyene, natamycin 5%. Amphotericin B, another polyene, was used before the development of natamycin; either one of them can be used as an initial therapy. [9] Other antifungal drugs that can be prepared for topical use include fluconazole,

voriconazole, miconazole, ketoconazole and posaconazole. Some studies were conducted to assess the efficacy of oral route of some antifungal drugs such as voriconazole, posaconazole and terbinafine which showed variable success against different fungal species. [2,10,11] Topical fluconazole via liposomes, a drug delivery system, was found to be successful especially against *Candida albicans* keratitis [12].

Though antifungal choices are vast, poor penetration and limited response to treatment are commonly encountered, that eventually lead to surgical intervention. Surgery include periodic debridement, conjunctival flap and therapeutic keratoplasty either lamellar or full-thickness. [13,14]

Many studies concerning intrastromal injection of antifungal drugs in cases of fungal keratitis were conducted over the past years on different groups of patients using different antifungal agents and comparing the results with those of other modes of drug administration. This study aims at evaluating the efficacy of intrastromal injection of antifungal drugs on proved fungal keratitis cases as an adjuvant route of administration of the antifungal drugs.

2. MATERIALS AND METHODS

Eighteen eyes of 18 consecutive patients admitted to Ophthalmology Department, Assiut University Hospital were analyzed prospectively during the period from March 2016 to November 2017.

2.1 Inclusion Criteria

1. Patients with fungal keratitis (as proved by full microbiological assessment including the identification of both budding yeast or hyphae on direct smear with wet mount potassium hydroxide *procedure and identifying fungal species after two weeks incubation on Sabouraud-Dextrose agar culture plates*).
2. No administration of antifungal agents within 3 days prior to participation in the study.

2.2 Exclusion Criteria

1. Non-fungal keratitis including bacterial, viral, acanthamoebic or non-infective keratitis.
2. Presence of vitritis/endophthalmitis (as proved by ophthalmic ultrasonography).

3. Antifungal therapy within 3 days prior to participation in the study.
4. Patient refusal to participate in the study.

2.3 Methods

All patients were subjected to the following:

- 1- Detailed history using a predesigned questionnaire.
- 2- Examination by slit lamp bio-microscopy to measure ulcers' maximum vertical and horizontal diameters, infiltrate depth and anterior chamber examination for the presence of any cells, flare or hypopyon and measuring its height.
- 3- Posterior segment assessment by ophthalmic ultrasonography to exclude the presence of any echoes suspicious of vitritis or endophthalmitis.
- 4- Informed consent was obtained from all cases. Patient's confidentiality was respected. Patient's refusal of participation in the study did not affect the quality of treatment they had received in the hospital.
- 5- Corneal scrapings were obtained for microbiological examination. as follows; topical anesthetic was instilled prior to corneal scraping. Then, corneal scraping was obtained by using a sterile surgical blade (no.11) by scraping the ulcer deeply at its base with caution, especially in the case of central corneal thinning.
- 6- Direct smear with wet mount potassium hydroxide procedure was done, in addition to culturing of the corneal scrapings on top of Sabouraud Dextrose agar plates, all of which were sent to Microbiology Department, Faculty of Medicine Assiut University to determine the presence of mold hyphae or budding yeast cells by examining direct smears under microscope and specifying the fungal species after incubating the culture plates up to two weeks (to exclude the presence of slow growing fungi).
- 7- Antifungal Drugs preparation for intrastromal injection:

- **Fluconazole 0.2% (2 mg/ml)**

This concentration is available without the need for preparation. (each 1ml contains 2mg)

- **Amphotericin B 5 to 10 µg/0.1ml**

To reconstitute 10 µg/0.1ml

Method: Prepare 5 mg/ml (as mentioned above) – take 0.2 ml solution and add to 0.8ml BSS. Now take 0.1 ml of this solution and add 0.9 ml BSS to create 0.1mg/ml equivalent to 10µg/0.1 ml. Use Immediately

- **Voriconazole 50 µg/0.1ml**

Method: From 1% solution Voriconazole, take 1ml, add to 19 ml ringer lactate to make 0.05mg/ml (50 µg/0.1ml).

- 8- Intrastromal injection (for proved cases of fungal keratitis) was performed in the operation theatre in complete aseptic conditions; the procedure was done in all patients as follows:

- Topical anesthetic was instilled.
- The drug was loaded in 30-gauge insulin syringe
- The needle penetrated the cornea obliquely at 30° - 45° with the bevel up in the mid stroma at the clear cornea around the edge of the infiltrate
- The drug was injected slowly in the stroma to induce edema, whose edge touches the edge of the infiltrate
- The injection was given in 3 to 5 divided doses (according to the size of the ulcer and infiltrate) to form a barrage around the lesion.

- 9- Topical natamycin 5% hourly was added to the ulcer regimen for all cases, in addition to moxifloxacin HCl 0.5% E.D. 5 times daily, cyclopentolate 1% E.D. tid, doxycycline HCl 100 mg tab bid for its anticollagenase effect.

Follow up of the patients was done on the first day, third day, one week, two weeks and one month post-injection.

More than one injection was done in selected cases on the 1st and/or 2nd weeks after the first injection based on individual circumstances such as ulcer size and response to intrastromal injection.

All ulcers were photographed before intervention and on the 30th day post-injection via smartphone-based digital imaging.

2.4 Statistical Analysis

Data entry and data analysis were done using SPSS version 23.0 (Statistical Package for

Social Science, version 23.0). Data were presented as number percentage, mean and standard deviation. Chi-square test and Fisher Exact test were used to compare between qualitative variables. independent sample t-test were used to compare quantitative variables between groups. P-value considered statistically significant when $P < 0.05$.

3. RESULTS

Table (1) shows clinical data in study group, with mean of time presentation 14.10 days. As regards mean value of size of ulcer 10.72 mm²,

also mean value of infiltration size was 14.34 mm² and mean value of depth of keratitis was 58%.

Table (2) shows relation between clinical data & Outcome in study group. There was significant difference ($P = .05$) between patients who had complete cure & incomplete cure with age, time of presentation, ulcer size and depth of keratitis. Also, there was highly significant difference ($P < 0.000$) as regards percent of ulcer size change. Regarding to infiltration size, there was non-significant difference ($P = .483$).

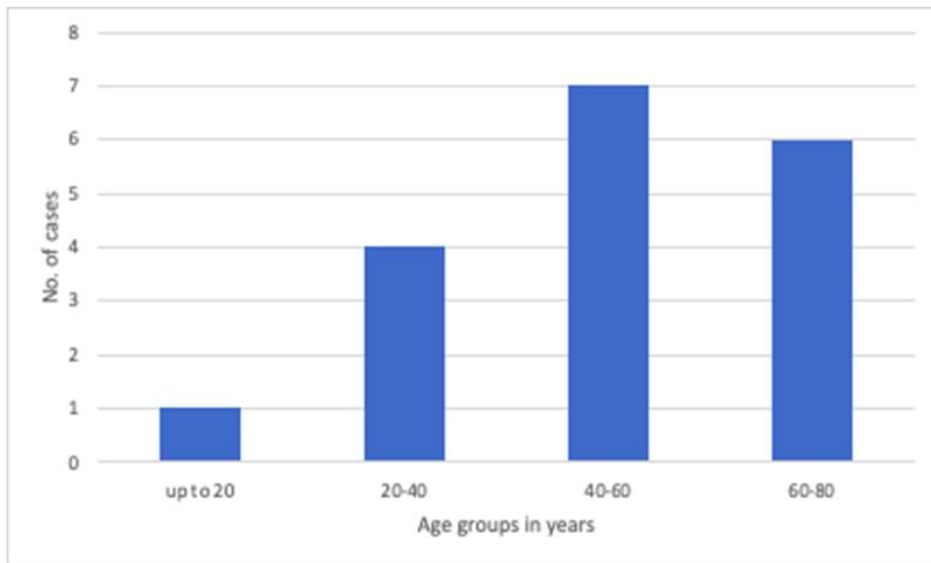


Fig. 1. Age distribution in study group

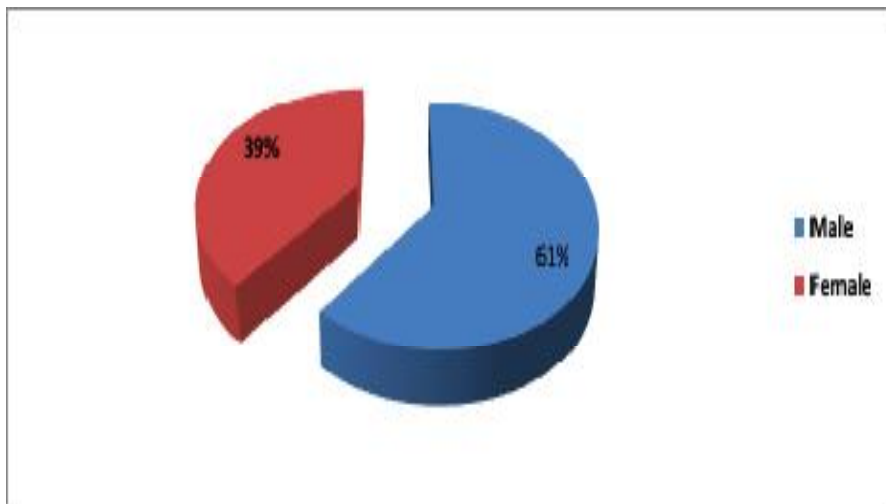


Fig. 2. Gender distribution in study group

Table 1. Clinical data in study group

Item	Descriptive “n=18”
1- Time of presentation(days)	14.10±8.23
2- Ulcer size(mm ²)	10.72±2.63
3- Infiltration size(mm ²)	14.34±3.14
4- Depth of keratitis (%)	58±3

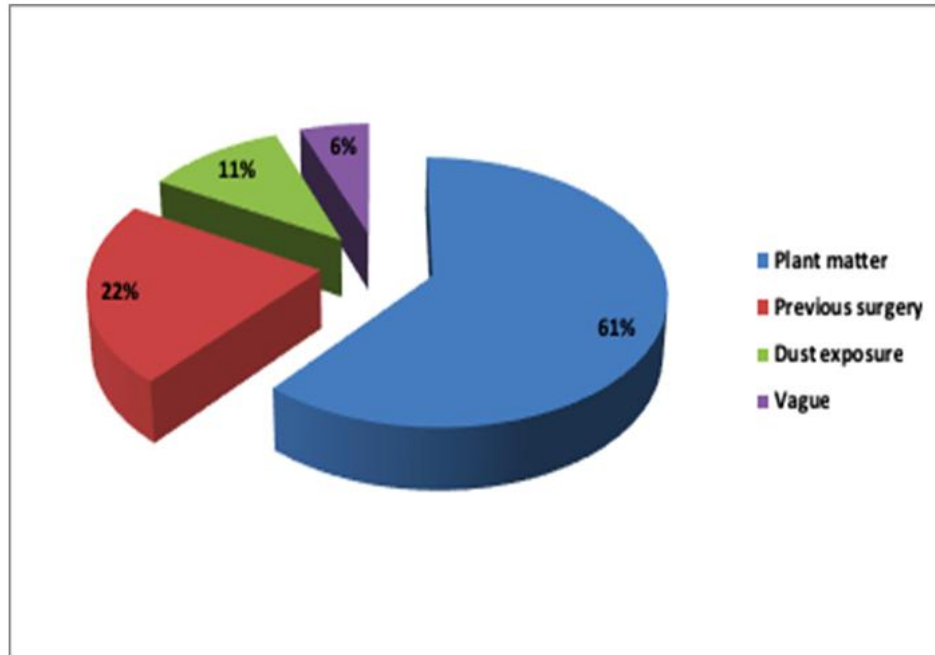


Fig. 3. Causative risk factors in study group

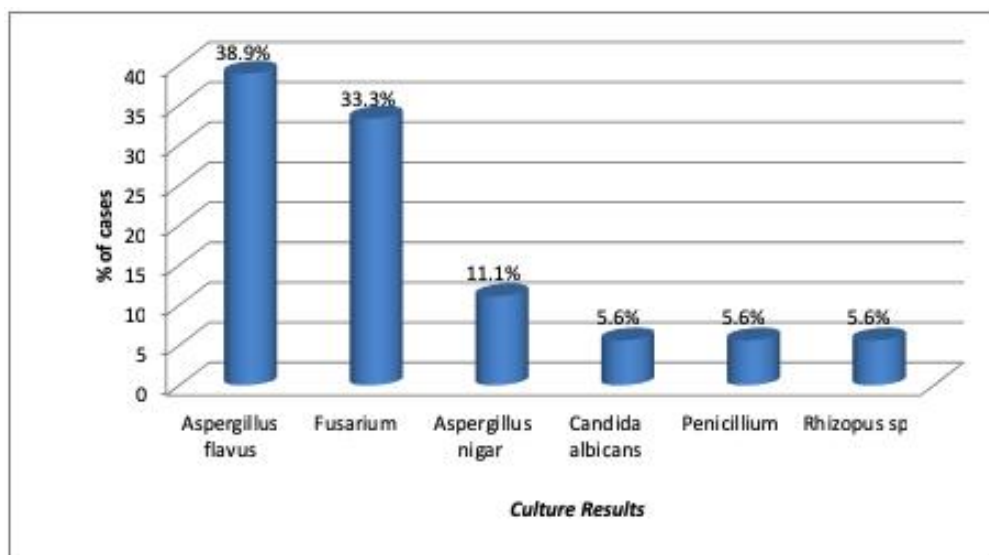


Fig. 4. Culture results in study group

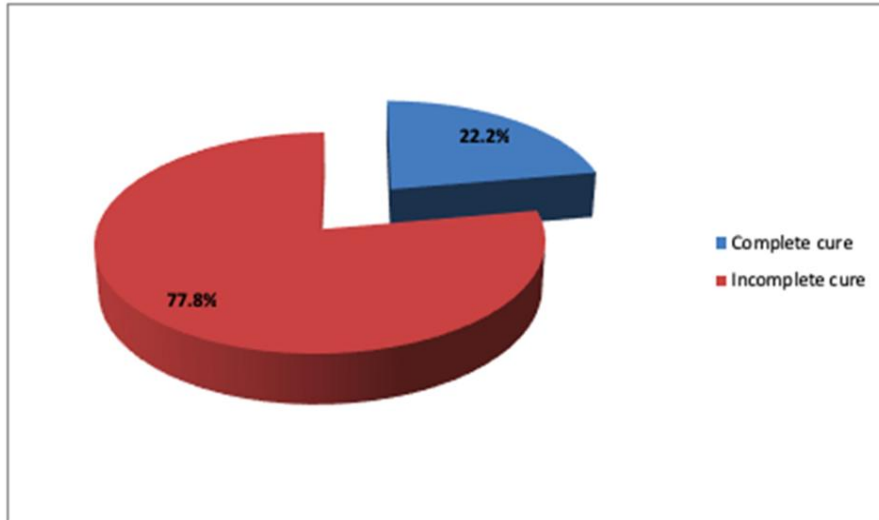


Fig. 5. Outcome in study group (after first injection)



Fig. 6. Shows corneal ulcer before scraping for smearing and culturing on the left, corneal ulcer after scraping in the middle and outcome after one week of one injection of fluconazole 0.2% resulting in complete cure with dense corneal opacity on the right

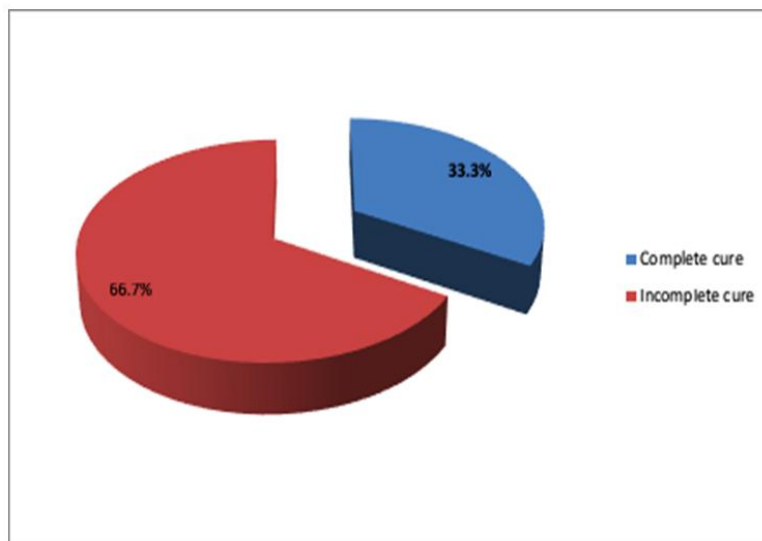


Fig. 7. Outcome in study group (after second injection)

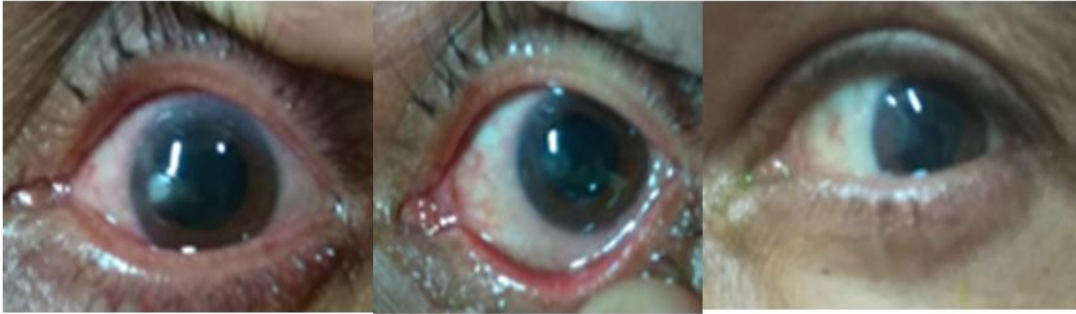


Fig. 8. Shows corneal ulcer before intrastromal injection on the left, resulting in incomplete cure after one week of first injection as shown in the middle and complete cure after 3 days of second injection on the right

N. B. Of the two cases that were injected thrice, none of them showed complete cure after the third injection

The figure shows relation between Culture & Outcome in study group. There was non-significant difference with P-value = .275 between type of culture and outcome of patients.

According to in vitro sensitivity results, Fluconazole 0.2% was used in 15 cases, Amphotericin B 5-10 µgm/0.1 ml was used in 2 cases and Voriconazole 50 µgm/0.1 ml was used in one case.

Descemetocoele was noted in two cases after injection, these cases were caused by *Candida albicans* and *Fusarium*, both cases showed deep infiltrate up to two-thirds of the corneal thickness.

Perforation was noted in one case that showed central thinning prior to intervention. *Aspergillus niger* was isolated from gentle corneal scraping, that was injected twice with 0.2% Fluconazole. Three days after the second injection, central perforation 0.5 mm in diameter with shallow AC was noted, that was managed by tissue adhesive (Histoacryl glue).

4. DISCUSSION

Fungal keratitis is a vision-threatening infectious disease. In general, the available choices of antifungal drugs are less than those of antibacterial drugs; besides, they have poorer tissue penetration. Therefore, the prognosis of fungal keratitis tends to be worse than bacterial keratitis as according to Niki, et al. [15]

In this study, patients included were (61.1%) male vs. (38.9%) female. This agrees with Yalaka Jayapal Reddy, et al., [16] who reported keratomycosis is more common in males (56.7%) than in females (43.3%). This can be explained owing to the fact that males, in agricultural Middle-East countries, are more vulnerable to trauma by plant matter, which is the most common causative risk factor for fungal keratitis as shown in this study, where trauma by plant matter was responsible for 61%, in comparison to other risk factors, such as previous surgery (22%) and dust exposure (11%).

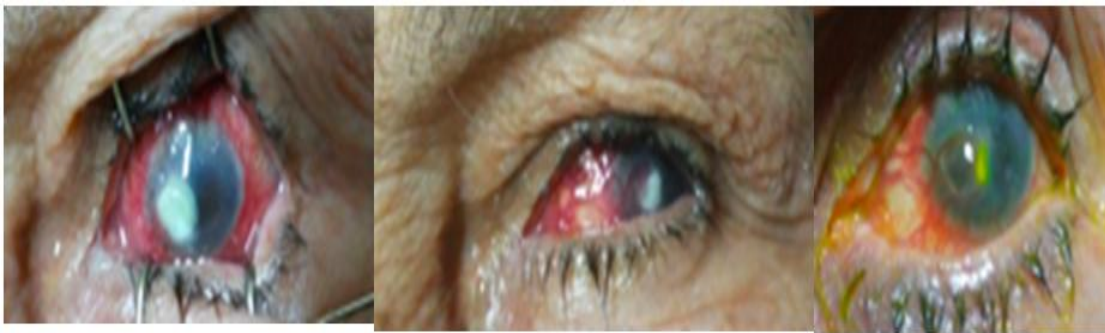
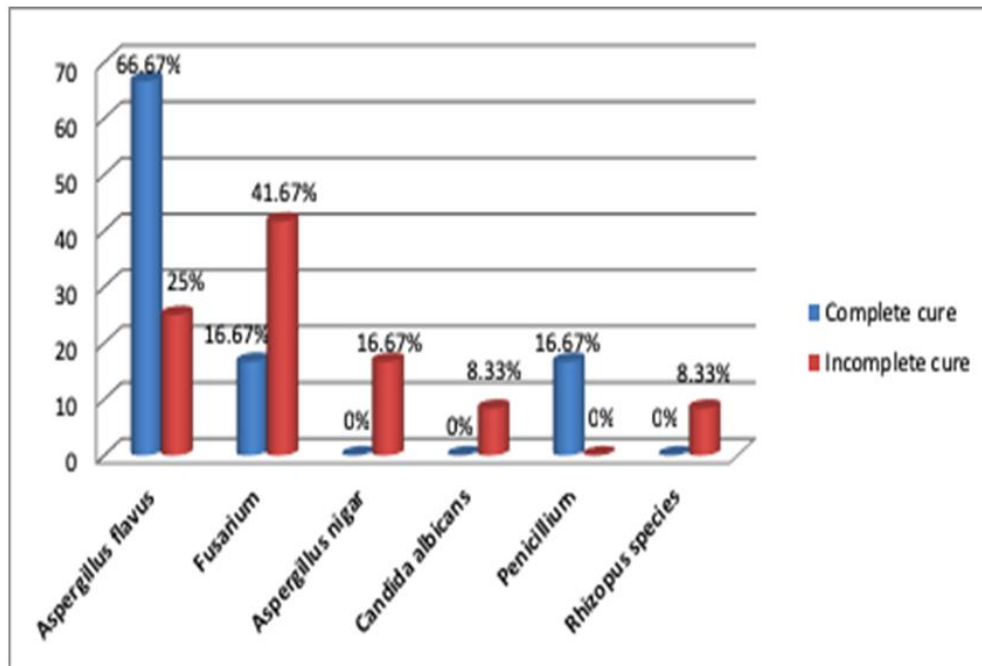


Fig. 9. Shows corneal ulcer before intrastromal injection on the left, incomplete cure after two weeks of first injection in the middle, incomplete cure with a resultant descemetocoele after one week of the second injection on the right

Table 2. Relation between clinical data & Outcome in study group

Item	Complete Cure "n=6"	Incomplete cure "n=12"	P-value
1- Age	34.02±16.22	56.35±13.90	P= .04*
2-Time presentation	9.25±4.57	15.48±8.63	P= .03*
3-Ulcer size	4.27±3.05	10.80±3.16	P= .01*
4-Infiltration size	9.04±6.58	15.85±3.59	P= .483
5-Depth of keratitis	0.45±0.02	0.62±0.04	P= .04*
6- Percent change of ulcer size	--	55.34±6.78	P< 0.000***

**Fig. 10. Percent of cases with complete cure and incomplete cure for each fungal species in this study**

Regarding the time needed for the healing of ulcers in this study, and following up for one month after intrastromal injection, there was change in ulcer size and infiltration size with percentage 55.34% in 12 patients who had incomplete cure and complete cure (100%) of ulcer in 6 patients. Five out of 6 cases of the complete cure showed rapid healing within one week. This agrees with Niki, et al., [15] who reported that one week after injection, corneal ulcers had healed and corneal infiltrates decreased. Also, this agrees with Xinying You, et al. [17] who reported that the healing time was 3–16 days (5.1 ± 3.1), and in 28 cases, the time was less than 7 days (51.9%) with four cases being 3 days in 54 eyes treated with lamellar keratectomy followed by intrastromal injection of fluconazole 0.2%.

Comparing patients with complete cure vs. patients with incomplete cure regarding time of presentation in this study, there was significant difference ($P < 0.05$) being lower in time of presentation in patients who had complete cure 9.25 days vs. 15.48 days in those with incomplete cure. This agrees with Maja Pauk-Gulić, et al., [18], who used topical and intrastromal injection of voriconazole combined with systemic fluconazole in treatment of fungal keratitis in corneal grafts.

All cases were followed up to one month after intrastromal injection, with 22.2% of cases showed complete cure and 77.7% of cases showed incomplete cure after first intrastromal injection, then 33.3% of cases showed complete cure and 66.7% of cases showed incomplete

cure after second injection with no further change in outcome after third injection. However, Yalaka Jayapal Reddy, et al., [16] whose follow-up time was up to 6 weeks after injection of thirty cases with voriconazole 50 µg/0.1 ml intrastromally, noting a faster reduction in corneal infiltrate size and complete resolution of ulcers with no adverse effects in 26 out of 30 cases (86.67%). Also, according to Xinying You, et al., [17], when followed up for more than 90 days, 89% (41 of 46 eyes) showed improvement in UCVA and 11% were unchanged. These higher results of complete cure than this study explains that longer follow-up time is necessary for cases of fungal keratitis even with intrastromal injection.

Three antifungal drugs were used in this study according to in vitro sensitivity results. They included fluconazole 0.2% which was used in 83.3% (n=15) of cases, amphotericin B 5-10 µgm/0.1 ml used in 11.1% (n=2) of cases and voriconazole 50 µg/0.1 ml in 5.56% (n=1) of cases. However, Sharma N, et al., 2013 [19] who used one agent only that was voriconazole 50 µg/0.1 ml intrastromally in comparison to topical voriconazole, concluded that intrastromal injection didn't offer any beneficial effect over topical therapy. This explains that depending on in-vitro sensitivity results may offer better way of deciding the more effective agents in intrastromal injection of antifungal drugs than using only one agent in managing all cases of fungal keratitis with different species and accordingly, different patterns of response to antifungal drugs.

In this study, six out of 18 cases yield *Fusarium* species, all of which were sensitive to fluconazole 0.2% according to in vitro sensitivity test. One case showed complete cure after single intrastromal injection in comparison to 5 cases that only showed incomplete cure; two of which showed decrease in ulcer size by more than 80% (88% and 89.5%), one showed regression better than 60% (66.3%) of ulcer size, and the remaining 2 cases showed regression less than 40% (9.4% and 28.5%). In other words, 3 cases of *Fusarium* keratitis showed great response, one case with moderate response and two cases with minimal response to intrastromal injection of fluconazole 0.2%. This agrees with Kalaiselvi G, et al., [20] who used voriconazole 50 µg/0.1 ml in 25 patients, where *Fusarium* species was responsible for 6 out of 7 of the failed cases, concluding that *fusarium* keratitis showed suboptimal response to intrastromal injection of Voriconazole 50 µg/0.1 ml. Also, this agrees with Nada, et al., [21] who showed failure

of treatment by combination of Amphotericin B 0.02 mg/ml and topical fluconazole in 7 cases of *Fusarium* keratitis.

The following complications of this technique were found in this study in comparison to other studies as follows:

- One case (5.56%) with *Aspergillus nigar* showed a small 0.5 mm central perforation 3 days after second intrastromal injection with fluconazole 0.2%, that was treated with tissue adhesive (Histoacryl glue) to seal the perforation. This agrees with Yalaka Jayapal Reddy, et al., [16] who showed 2 cases (10%) experienced perforated ulcer after 2 weeks of treatment with intrastromal injection of voriconazole 50 µg/0.1 ml. Those cases underwent Keratoplasty.
- Two cases were complicated by corneal thinning (descemetocoele) after intrastromal injection. These cases showed deep infiltrate more than two-thirds of corneal thickness. This agrees with Xinying You, et al., [17] who reported one case of corneal thinning with vascularization after lamellar keratectomy followed by intrastromal injection of fluconazole 0.2%.

The limitations of this study are the small number of the included eyes and a short follow-up time, and a clinical trial of a large series of patients with longer follow-up time, conducted in a randomized controlled manner, is desirable.

5. CONCLUSION

Advanced cases of fungal keratitis show good response to intrastromal injection of antifungal drugs when combined with the traditional topical route. Earlier intervention before occurrence of corneal thinning shows even better results with less incidence of possible complications.

CONSENT

A written informed consent was obtained from all patients participated in the study after explaining the whole procedure and possible complications.

ETHICAL APPROVAL

All authors, hereby, declare that approval was obtained from the Medical Research Ethics Committee of the Scientific Research, Faculty of

Medicine, Assiut University that adhered to the tenets of the Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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