

# Different Modalities of Antifungal Agents in the Treatment of Fungal Keratitis: A Retrospective Study

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Received date: November 08, 2016; Accepted date: January 31, 2017; Published date: February 03, 2017

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## Abstract

**Purpose:** To evaluate the efficacy of different modalities of antifungal agents in the treatment, clinical features and therapeutic outcome of fungal keratitis

**Design:** Retrospective observational case series.

**Methods:** The study reviewed 251 eyes of 246 patients treated for moderate and severe fungal keratitis in the period from 2010 to 2015. The diagnosis of fungal keratitis based on the clinical characteristics features of fungal keratitis beside laboratory diagnosis. The antifungal drugs were determined according to the commercial availability at the time depending on the clinical features, also to some extent to the laboratory diagnosis. Ten different modalities of antifungal agents beside antibacterial agents and cycloplegic drugs were used.

**Results:** Among the total treated 251 eyes, 194 eyes (77.29%) showed complete healed ulcers. But 121 eyes were treated by five groups of combined therapy of antifungal agents achieving healed ulcer in 97 eyes (80.16%). The study reported 10 groups of different modalities of antifungal agents. The highest curative rate was 88.46% in cases treated by combined therapy of corneal intrastromal injection of amphotericin B beside topical fluconazole with mean duration of healing ( $25.43 \pm 4.09$  days). The second rate was 84% in a combination of topical natamycine and subconjunctival injection of amphotericin B with mean duration of healing  $27.95 \pm 3.46$  days. The shortest duration of healing was  $24.83 \pm 4.39$  days in cases treated by a combination therapy of corneal intrastromal injection of voriconazole beside topical natamycine with curative rate 82.14%.

**Conclusions:** The use of a combined therapy of antifungal agents achieved the best treatment modality in cases of fungal keratitis especially the combination of intrastromal injection of antifungal agents with topical one according to curative rate and duration of healed ulcers in cases of moderate and severe fungal keratitis.

**Keywords:** Fungal keratitis; Antifungal agents; Corneal intrastromal injection

## Introduction

Fungal keratitis is still refractory and vision-threatening disease. Although the choice of antifungal drugs has been increasing, the challenge in management of fungal keratitis based on the different fungal pathogens virulence among filamentous, yeast species and host response [1].

The drug delivery into the corneal tissues and identification of fungal pathogens play important roles in management of fungal keratitis [2]. Although various genera and species of fungi cause fungal keratitis, the drug susceptibility patterns make important evidence useful for fungal keratitis treatment [3].

Many antifungal agents were used by different authors according to the commercial availability in their countries including the two major groups of antifungal agents: Azole group and polyene group. Still voriconazole from the azole group and natamycine from the polyene group play the most important role in the treatment of fungal keratitis by different routes of administration either topical, intracameral or

intrastromal injection [4-8]. One study by FlorCruz and Evans [9] reported 12 trial of medical treatment of keratomycosis in different countries, they stated variation in the results between different antifungal agent such as natamycine, amphotricin B, voriconazole, fluconazole and itraconazole. The efficacy of the drugs depends on the route of administration and virulence of the pathogens. Also surgical treatment can play a role to prevent visual impairment [10].

The combined therapy of antigugal agents between azole and polyne groups achieved best results in the treatment of cases with fungal keratitis [1,11].

Different fungal pathogens were detected by laboratory investigation including yeast and filamentous fungi such as candida, *alternaria*, *parapsilosis*, *penicillium*, *curvularia*, *scedosporium*, *aspergillus* and the most virulent was *fusarium* [3,12-14].

## Methods

This observational study reviewed 251 eyes of 246 patients treated for moderate and severe fungal keratitis according to Richard et al. [15] of clinical grading of corneal ulcers, mild (less than 2 mm width and less than one third depth), moderate (2-6 mm width and more

than one third depth) and severe (more than 6 mm width, or with hypopyon). The study was performed at Ophthalmology Department, Faculty of Medicine, Zagazig University during March 2010 to May 2015. The study was performed according to WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects. The diagnosis of fungal keratitis based on the clinical characteristics features including:

### History taking

The onset of the ulcer, course and duration. Age, occupation with special consideration for jobs related to agriculture field, systemic diseases especially diabetes mellitus and corticosteroid use or any immunity debilitating diseases, plant or vegetable trauma, surgical trauma, contact lens wearing history, previous corneal disease, ulcer, opacity and history of resistant corneal ulcer.

### Ophthalmological examination

Full examination of the eye specially the cornea in three dimensions pattern and searching of the characteristic features of keratomycosis such as: thick elevated lesion, feathery edges, surrounding satellites, area of corneal staining by fluorescence is less than area of infiltration, irregular hypopyon and surrounding corneal gutter.

Laboratory profile was done for cases including: complete blood picture, liver and kidney function tests, blood glucose, some special tests for indicated cases such as rheumatoid arthritis and thyroid eye disease or other diseases under corticosteroid thereby. Also corneal specimen was taken and applied for direct corneal smear using gram and giemsa stains beside culture on sabourad dextrose agar media for fungal pathogens and nutrient agar for bacterial pathogens. Culture was incubated for 14 days to get results.

Some cases were treated empirically by antifungal agents depending on the history and clinical picture beside some precautions that prevent taking a corneal specimen such as corneal thinning or desmatocele.

Ten Different modalities of antifungal agents were used arranged by time of use from the recent to the older use:

**Group 1:** Combined once intrastromal injection voriconazole 50 µg in 0.1 ml and topical natamycine eye drops 5%.

**Group 2:** Combined once intrastromal injection of amphotericin B 20 µg in 0.1 ml and topical fluconazole eye drops 2%.

**Group 3:** Combined topical amphotericin B 0.3-0.4 mg-ml and subconjunctival injection of fluconazole 2%.

**Group 4:** Combined topical natamycine 5% and subconjunctival amphotericin B 1 mg.

**Group 5:** Twice intrastromal injection of amphotericin B 50 µg in 0.1 ml.

**Group 6:** Topical natamycine 5% eye drops.

**Group 7:** Topical amphotericin B eye drops 0.3-0.5 mg in ml.

**Group 8:** Topical fluconazole 2% eye drops.

**Group 9:** Combined topical natamycine 5% and fluconazole 2% eye drops.

**Group 10:** Topical itraconazole 1% eye drops.

Topical antibiotic drugs (Tobramycine 0.3%, gatifloxacin 0.3% and moxifloxacin 0.5%) and cycloplegic drugs (atropine sulphate 1%) were added to the antifungal agents in all groups, all these modalities were recorded and follow up of cases was done each 48 h in the first 2 weeks and weekly in the following weeks up to 6 months for the progress of the ulcer detecting the healing criteria as size of the ulcer, corneal staining, absence of hypopyon and ciliary injection of the cornea and pain. The duration of healing of the ulcers was recorded and failure of treatment up to 21 days depending on the progress of the ulcer mentioned before was recorded as failure and shifted to other modalities. The side effects of the drugs used were detected such as burning sensation, corneal melting and conjunctival necrosis.

Interpretation, correlation between different treatment modalities of the results was performed.

### Results

The demographic data of patients in all groups revealed that, the age of the patients varies from (43 ± 7.2) years in group 10 to (57.3 ± 11.33) years in group 9. Among 246 patients there were 158 male (64.23%) and 88 female (35.77%) with sex ratio 1.8 (Table 1).

Mean duration in days	Healed ulcers in eyes		Age in years	No. of patients			No. of eyes	Groups
	%	No.	Mean age	total	F	M		
24.83 ± 4.39	82.14	23	56.4 ± 14.32	27	12	15	28	1
25.43 ± 4.09	88.46	23	53.41 ± 7.05	26	8	18	26	2
28.55 ± 3.89	76.92	20	47.5 ± 8.12	26	7	19	26	3
27.95 ± 3.46	84	21	50.9 ± 12.08	25	4	21	25	4
29.83 ± 5.12	60	6	46.8 ± 13.34	10	4	6	10	5
28.45 ± 4.02	72.09	31	52.4 ± 10.06	41	15	26	43	6
35 ± 6.93	80.56	29	55.5 ± 9.07	35	18	17	36	7
36.19 ± 5.91	81.25	26	56.45 ± 16.9	31	12	19	32	8
37.3 ± 6.33	62.5	10	57.3 ± 11.33	16	8	8	16	9
43.2 ± 4.21	55.56	5	43 ± 7.2	9	0	9	9	10
	77.29	194		246	88	158	251	Total

**Table 1:** Demographic data and duration of healed ulcers in all groups.

The predisposing factors for fungal keratitis revealed that, trauma was mostly presented in 94 eyes (37.45%) either plant, surgical or other types, and resistant corneal ulcers in 85 eyes (33.86%) presented by either exciting epithelial defect in immune deficient patient or topical corticosteroids use.

Among 251 eyes included in the study, there were 53 (21.11%) eyes treated empirically by antifungal agents depending on the clinical picture, 198 (78.89%) eyes were applied for laboratory fungal culture on sabaroud dextrose agar media.

Culture results revealed that 86 eyes (43.43%) were *Candida*, 24 eyes (12.12%) were *Aspergillus*, 18 eyes (9.09%) were *Alternaria*, 14 eyes (7.07%) were *Penicillium*, 7 eyes (3.53 %) were *Scedosporium*, 7 eyes were (3.53%) *Fusarium* and 42 eyes (21.21%) revealed negative results after 14 days incubation, so that the positivity of culture results constituted 78.79% of corneal specimens. Most of culture results 135 cases (68.1%) resulted between 6-8 days of culture incubation.

Direct corneal smear was performed in 167 eyes using giemsa stain in 45 eyes and gram stain in 122 eyes, fungal pathogens were detected only in 99 eyes with a positivity rate (59.28%), yeast spores were detected in 62 eyes (50.81%) and filamentous fungi were detected in 60 eyes (49.19%).

Among 10 different treatment modalities by antifungal agents of 251 eyes, 194 eyes (77.29%) showed complete healed ulcers, 121 eye were treated by five different groups of combined therapy of antifungal agents resulting in healing ulcers in 97 eyes (80.16%) with a mean duration (24.83 ± 4.39) to (37.3 ± 6.33) days, 130 eyes were treated by also different groups of a monotherapy of antifungal agent resulting in healing of 97 eyes (74.61%) with longer duration of healing from 28.45 ± 4.02 to 43.2 ± 4.21 days.

There were 2 groups of combined antifungal therapy resulting in highest curative rate and shorter healing duration of the ulcers, group 2 of combined intrastromal injection of amphotericin B and topical fluconazole eye drops, it achieved the highest curative rate (88.46%) among 26 eyes with mean duration of healing of the corneal ulcers (25.43 ± 4.09) days, and group 1 of combined intrastromal injection voriconazole and topical natamycine eye drops achieved the shortest mean duration of healing (24.83 ± 4.39) days with curative rate (82.14%) among 28 treated eyes, this combination achieved high success rate in *Fusarium* infections (most virulent fungal pathogens) which had recorded treatment failure in other groups.

With regards to the other groups, different curative rate between 55.56% in group 10 of topical itraconazole among only 9 eyes to 81.25% in group 8 of topical fluconazole eye drops among 32 eyes Table 1.

Tests of significance did not applied between the groups due to different number of eyes in each group. So the comparison between groups did not achieved high accuracy of analysis but the study to some extent can report some useful results rather than significant statistical relations.

Best corrected visual acuity among 194 healed eyes reported that 6 eyes (3.09%) had vision of light perception (PL), 71 eyes were hand motion (HM) vision (36.59%), 46 eyes (23.71%) between 0.01 to 0.05 vision. Also 71 eyes (36.6%) were subjected to visual correction by glasses including 34 eyes (17.52%) between 0.1 to 0.3 vision and 37 eyes (19.07%) between 0.4 to 0.5 vision (Table 2).

Among 194 healed eyes, the records reported only 16 eyes were subjected to penetrating keratoplasty.

The side effects of the drugs used are as follows: burning sensation were detected in 10 cases in group 7 of topical amphotericin B users and 5 cases in group 6 of topical natamycine users.

Group s	No. of healed eyes	Visual acuity									
		PL		HM		0.01-0.05		0.1-0.3		0.4-0.5	
		No	%	No	%	No	%	No	%	No	%
1	23	0	0	5	21.7	7	30.4	4	17.4	7	30.4
2	23	1	3.8	6	26	5	21.7	4	17.4	7	30.4
3	20	0	0	7	35	6	30	3	15	4	20
4	21	2	8	9	42.9	3	14.3	4	19	3	14.3
5	6	1	10	2	33.3	1	16.6	2	33.3	0	0
6	31	0	0	9	29	8	25.8	4	12.9	10	32.3
7	29	1	2.8	13	44.8	8	27.6	4	13.8	3	10.3
8	26	1	3.8	12	46.1	4	15.4	6	23	3	0
9	10	0	0	5	50	3	33.3	2	20	0	0
10	5	0	0	3	60	1	20	1	20	0	0
Total	194	6	3.09	71	36.59	46	23.71	34	17.52	37	19.07

Table 2: Visual outcome in healed ulcers in all groups.

## Discussion

Cases of fungal keratitis are considered as challenging eye problem in many countries especially agriculture ones as it was more frequent with plant trauma reported by the present study and Cheikhrouhou et al. [12] reporting the age between 45 and 50 years which was similar with the present study that reported age between (43 ± 7.2) and (57.3 ± 11.33) years as this age was more susceptible for fungal infections related to their job. Also sex ratio may play a role in susceptibility for fungal keratitis as the present study reported that most cases were male with a ratio of 1.79 nearer to the study of Cheikhrouhou et al. [12] 1.58, in spite of the study of Vanzini Zago et al. [13] in Mexico who reported a ratio 4. The difference in ratios may be related to difference in the nature of the occupations in many countries depending on age and sex distribution.

With regards to the predisposing factors for fungal keratitis, the present study reported trauma in (37.45%) of eyes similar to Vanzini Zago et al. [13] with 36% in spite of Cheikhrouhou et al. [12] with 61.6%, all these studies proved that trauma especially plant or vegetable ones plays very important role in occurrence of fungal keratitis. So the age and occupation of patients were related to the fungal infection of the cornea especially in agriculture countries.

Most incriminated fungal pathogens included yeasts and filamentous fungi, the present study reported by direct corneal smear nearly equal proportion between them (62 eyes) and (60 eyes) and by culture, the study revealed positive samples in (78.79%) among the collected samples, as 86 eyes (43.43%) were *Candida*, 24 eyes (12.12%) were *Aspergillus*, 18 eyes (9.09%) were *Alternaria*, 14 eyes (7.07%) were *Penicillium*, 7 eye (3.53%) were *Scedosporium*, 7 eye were (3.53%) *Fusarium* and 42 eyes (21.21%) revealed negative results after

14 days of incubation, in comparison to Sunada et al. [3] who detected fungal pathogens in 72 (50.7%) out of 142 samples. The major isolates were *Fusarium* (18), *Candida parapsilosis* (12), *Candida albicans* (11) and *Alternaria* (6), But Cheikhrouhou et al. [12] reported high percentage (93%) of positive cultures. Filamentous fungi form the major etiologic agents (83%): *Fusarium* species (49% with *F. solani*), *Aspergillus sp.* (22%), *Alternaria* (5%), *Scedosporium* (2%); and non-identified mold in (5%). Yeasts were identified in 17% of cases. Also Nielsen et al. [16] reported 52% with *Candida*, 20% with *Fusarium*, 16% with *Aspergillus* and 12% with mixed filamentous fungi. There was a wide difference between the studies about the culture results of corneal specimens; it may be due to the difficulties in incorporation of the pathogens into the specimen as it needs deep corneal scrapping to reach the deep layers of the corneal stroma where the pathogens were deeply penetrating the cornea.

The present study reported 10 different treatment modalities by antifungal agents, among 251 eyes, 194 eyes (77.29%) showed complete healed ulcers, 121 eye were treated by five groups of combined therapy of antifungal agents achieving healed ulcer (80.16%) with a short duration of healing from (24.83 ± 4.39) to (28.55 ± 3.89) days, 146 eyes were treated by monotherapy of antifungal agent achieved healing in (73.28%) with longer duration of healing from (28.45 ± 4.02) to (43.2 ± 4.21) days, so that the combined antifungal therapy between the polynes and azole groups can achieved good treatment modality in such cases as reported by Al-Hatmi et al. [11] who agreed this concept of such combination due to the broad spectrum activity of the combined antifungal therapy.

Intrastromal injection of amphotericin B as reported by Garcia-Valenzuela and Song [6] and Intrastromal injection of voriconazole as reported by Guber et al. [16] and the present study agreed all together that this route of administration of antifungal agent can achieved the less side effects and the shortest duration of healing among cases of fungal keratitis, as this route of administration making the antifungal agents invading directly the fungal pathogens deeply situated in the corneal tissues. Also voriconazole was considered one of the best antifungal agents proved by Guber et al. [17] and Solanki et al. [4] and also the present study revealed that this drug was highly efficient against *Fusarium* species. It was considered the most potent antifungal agent against both yeasts and filamentous fungi by either *in vitro* or *in vivo* susceptibility; also it was the less toxic drug for the corneal tissues.

The fungal pathogens tend to penetrate deeply in the corneal tissues resulting in deep keratitis and hence visual impairment after cure, as the present study reported 6 eyes (3.09%) had vision of light perception, 71 eyes were hand motion vision (36.59%), 46 eyes (23.71%) between 0.01 to 0.05 vision. Also, Inoue et al. [1] reported that visual impairment was the main outcome following fungal keratitis due to the dense corneal opacity as they reported 42.9% of cases was less than 20/200. Also the present study reported 16 eyes were subjected to penetrating keratoplasty aiming to treat this resulted visual impairment.

## Conclusions

Medical intervention in fungal keratitis by different modalities stated that the use of a combined therapy of antifungal agents achieved the best treatment modality in cases of fungal keratitis especially the combination of intrastromal injection of antifungal agents with topical one according to curative rate and duration of healed ulcers in cases of

moderate and severe fungal keratitis. Voriconazole was one of the best antifungal agents especially in management of resistant cases with *Fusarium* species.

## Conflict of Interest

The authors declare no conflict of interest.

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