*Original Article*

# Combined Application of 5% Natamycin and 0.2% Fluconazole for the Treatment of Fungal Keratitis

Hua Gong[1,*], Xiangming Gong[2]
*1 Liwan Hospital of Guangzhou Medical College, Guangzhou 510170, China*
*2 Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou 510060, China*

**Abstract**

**Purpose**: To observe the clinical efficacy of combined use of 5% natamycin and 0.2% fluconazole for the treatment of fungal keratitis.

**Methods**: A total of 65 cases diagnosed with fungal keratitis by direct smear and/or fungus culture from January 2010 to January 2013 were enrolled in this study. The duration from the onset of symptoms to admission to our ophthalmic center ranged from 9 to 90 d (mean 29.5±19.1 d) in the severe group, which significantly differed from the 7 to 36 d (mean 16.6±7.1 d) in the non-severe group ($P$<0.01). All cases were divided into non-severe and severe groups based on the degree of corneal inflammation. All cases were treated with topical use of 5% natamycin and 0.2% fluconazole once per hour. The same clinical and examination protocols were adopted for both groups.

**Results**: In the non-severe group, 23 of the 24 patients (95.8%) were healed, and 1 (4.2%) showed treatment efficacy. In the severe group, 12 of 41 patients (29.3%) were healed, 11(26.8%) showed clinical efficacy, and 18(43.9%) showed no efficacy. The patients between two groups significantly differed in terms of efficacy ($P$<0.01).

**Conclusion**: Combined use of 5% natamycin and 0.2% fluconazole is efficacious in treating fungal keratitis, especially mild or moderate infections. (***Eye Science 2013; 28:84-87***)

**Keywords**: fungal keratitis; natamycin ; fluconazole; treatment

In the recent decade, the incidence of fungal keratitis in China has shown a continuous increase, with *Fusarium sp.* and *Aspergillus sp.* as the major pathogens[1-4]. This is probably a result of the abuse of antibiotics and hormones and improper prevention and handling of ocular trauma, *etc*. Medicine administration seldom yields a desirable efficacy against fungal keratitis. Moreover, delayed diagnosis and treatment worsens the severity of this disease and increases the difficulty of treatment, leading to poor efficacy, a high rate of blindness, and even loss of the eyeball. Consequently, more attention should be diverted to enhance the cure rate and decrease rate of blindness due to fungal keratitis. Combined use of medicines has been frequently adopted to elevate their antifungal activity. The combined administration of natamycin and fluconazole were utilized to treat fungal keratitis in the present study.

## Materials and methods

### General information

The subjects enrolled in this investigation were admitted to Zhongshan Ophthalmic Center or transferred from Liwan Hospital of Guangzhou Medical College between January 2010 and January 2013. Informed consent was obtained from all participants. Sampling was performed at the corneal ulcer, observed with a microscope (KOH examination), and samples were subjected to fungal culture. Sixty-five patients were diagnosed with fungal keratitis. Among all patients, 49 were male and 16 female (sex ratio: 3:1), aged from 17 to 79 years, (50.8±13.9 years on average); 45(69.2%) were aged from 35 to 59 years and 20 (30.8%) were aged from 15 to 34 or from 60 to 79 years. Occupation: 46(70.8%) patients were peasants and the remaining 19(29.2%) were decoration workers or self-employed *etc*. Thirty-six cases had a definite history of ocular trauma including 11 with plant foreign bodies, 14 with metal, stone/sand particles, cement, or porcelain fragments, 11 with dust in the eyes or ocular discomfort caused by constant rubbing, and 29 with unknown reasons. All cases had unilateral trauma.

**DOI**: 10.3969/j.issn.1000-4432.2013.02.007
* **Corresponding author**: Hua Gong, E-mail: hngz.02059@163.com

**Grade of keratitis**

Based on the depth of corneal inflammation infiltration, size of the ulcer, and hypopyon, the keratitis was graded into non-severe and severe groups. Non-severe group: corneal ulcer inflammation infiltrated into the shallow and medium layers, ≤2/3 of corneal stroma thickness, ulcer diameter ≤6 mm. No severe or superficial reactions were noted in the anterior chamber. Severe group: corneal ulcer inflammation infiltrated into the deep layer, > 2/3 of corneal thickness or full-thickness cornea, ulcer diameter > 6 mm, and complicated with hypopyon.

**Treatment method**

All patients received combined use of 5% natamycin and 0.2% fluconazole eye drops, once per hour. During the 1st administration, fluconazole was initially given and 15 min later natamycin was delivered, allowing for sufficient time for the natamycin to adhere to the ulcer surface. The drops were administered once an hour, 12~13 times daily. Fluconazole ointment was daubed during the night and atropine was given for mydriasis as necessary. The severe patients took oral itraconazole (100 mg) twice daily. For those patients having a deteriorated state of an illness, enlarged ulcer, deepening infiltration, or corneal perforation following 7 to 10 d treatment, surgical methods including corneal transplantation were considered and timely performed.

**Efficacy evaluation**

Healing: corneal ulcer was healed, negative for fluorescence dye, stromal inflammation infiltration was basically recovered, scar formation was evident, hypopyon was absorbed, and the eye was negative for the Tyndall effect. Efficacious: corneal ulcer or corneal inflammation infiltration was alleviated and hypopyon was decreased or completely absorbed. Inefficacious: corneal inflammation remained the same or was aggravated, no changes in hypopyon, deteriorated corneal ulcer and corneal perforation were evident.

**Statistical analysis**

The data were analyzed by a *chi*-square test and *t*-test. $P< 0.01$ was considered as statistically significant.

## Results

**Laboratory examination**

Direct smears were obtained from 48 patients (73.8%) and were observed with a microscope (KOH examination). Of these, 40 cases were positive for fungal hyphae (positive rate 83.3%) and 8 were negative. However, these 8 cases were positive for fungal culture and fungal growth was noted. In total, 51 patients underwent corneal scraping at the ulcer site, and 47 had fungal growth (positive rate 92.2%) while 4 had none (negative rate 7.8%). The fungal species identified were as follows: 13 cases of *Fusarium spp.* (27.6%) including 10 *Fusarium solani*, 1 *Curvularia sp.*, 1 *Fusarium moniliforme* and 1 *Fusarium oxysporum*; 12 of *Aspergillus spp.* (25.5%) including 6 *Aspergillus flavus*, 2 *Aspergillus terreus*, 2 *Aspergillus fumigatus* and 2 *Aspergillus niger*; 7 *Helminthosporium gramineum*, 6 *Mucorales*, 4 *Curvularia sp.*, 3 *Penicillium*, 1 yeast-like fungus, and 1 unknown. Histological examination revealed fungal hyphae in 1 case.

**Treatment result**

As Table 1 shown, the overall treatment efficacy significantly differed between two groups ($\chi^2$ = 12.46, $P$ <0.01). In the severe group, 18 patients (43.9%) had no efficacy, 10 receiving corneal transplantation, 1 conjunctival flap cover and 7 ocular enucleation or eyeball enucleation due to corneal perforation or endophthalmitis complication.

In the non-severe group, the time starting from onset to admission to Zhongshan Ophthalmic Center was 7 to 36 d, 16.6±7.1 d on average, while this time was 9 to 90 d, 29.5±19.1 d in the severe group. Statistical significance was noted between two groups ($t$= 3.17, $P$< 0.01).

**Table 1** Comparison of treatment outcomes between two groups

| Group | Number of cases ($n$) | Healing ($n$/%) | Efficacious ($n$/%) | Inefficacious (n/%) | Overall efficacy rate (%) | Course of treatment (d) |
|---|---|---|---|---|---|---|
| Non-severe | 24 | 23(95.8%) | 1(4.2%) | 0 | 100% | 16~75(36.4±14.3) |
| Severe | 41 | 12(29.3%) | 11(26.8%) | 18(43.9%) | 56.1% | 21~67(39.0±17.4) |

## Discussion

Recent studies conducted in the northern, central,

**Table 2** Correlation between main pathogenic fungi and therapeutic efficacy

| Pathogenic fungal strains | *Fusarium* | *Aspergillus* | *Mucorales* | *Helminthosporium gramineum* | *Curvularia* | *Penicillium* |
|---|---|---|---|---|---|---|
| Total number | 13 | 12 | 6 | 7 | 4 | 3 |
| Efficacious ($n$) | 8 | 10 | 4 | 1 | 2 | 3 |
| Efficacy rate (%) | 61.5% | 83.3% | 66.6% | 14.3% | 50.0% | 100% |

**Table 3** Visual acuity before and after medicine administration

| | Visual acuity | 0.1~0.4 | 0.02~0.09 | Fingers counting | Hand movement to light perception | No light perception |
|---|---|---|---|---|---|---|
| Number of case | Before treatment | 0 | 4(6.2%) | 20(30.8%) | 41(63.1%) | 0 |
| ($n$/%) | After treatment | 18(37.0%) | 15(23.1%) | 13(20.0%) | 12(18.5%) | 7(10.8%) |

and southern China indicated that the incidence of fungal keratitis has constantly increased in the past decade[1–4]. Lixin Xie et al. reported that fungal keratitis accounted for 61.9% of hospitalized infectious corneal ulcers, highlighting the highest prevalence of fungal keratitis among cases of infectious corneal ulcer in the North of China[3]. The major pathogenic fungi have been reported to be *Fusarium* followed by *Aspergillus*, *etc*[1–4]. In Guangdong province, where the climate is humid and warm, a variety of fungi are likely to grow and proliferate. The incidence of fungal infection will be significantly higher compared with other regions if scant attention was paid to public and individual hygiene. The number of cases in this study was relatively small, 13 (27.6%) cases with *Fusarium* and 12 (25.5%) *Aspergillus* were analyzed, which were the primary pathogenic bacteria of fungal keratitis. *Helminthosporium gramineum* and *Mucorales* (27.6% in total) ranked the second and followed by *Curvularia* and *Penicillium*, which were consistent with previous findings[1,4]. These phenomena reflected that the primary pathog enic fungi of fungal keratitis still belong to *Fusarium* and *Aspergillus spp*. However, the ranking of alternative pathogenic fungi changes and the species of infectious fungi become intricate.

At present, administration of topical ocular drops serves as the primary treatment for fungal keratitis and the medicine used must be highly efficacious and yield low toxicity. Previously, amphotericin B was frequently applied for the treatment of fungal keratitis, but it has been gradually substituted by natamycin due to high toxicity and other limiting factors[5]. Natamycin acts as a broad-spectrum antifungal agent against *Candida*, *Fusarium*, *Curvularia*, *and Penicillium*, *etc*. The sensitivity of *Fusarium* towards natamycin was as high as 93.4%[6]. Natamycin eye drops are stable and cause no irritation or toxicity. Similar to amphotericin B, natamycin has poor penetration into the corneal epithelia. However, the injured epithelial layer in patients with fungal keratitis provides access to the medicine molecular penetration. In addition, the binding between natamycin and corneal tissues prolongs the contact time, reduces the dilution effect of conjunctival sac tears[5], and increases the antifungal concentration of natamycin within the cornea. Kalavathy et al. administered natamycin eye drops once an hour for the treatment of severe fungal keratitis and the cure rate was 61.5%[5]. In the present study, 41 of 65 (63%) cases have severe fungal keratitis with a mean time interval of 29.5±19.1 d from onset to admission to hospital, significantly differing from their counterparts in the non-severe group ($P< 0.01$). Since the majority of cases have severe fungal keratitis, the use of 5% natamycin and 0.2% fluconazole is combined with the aim of improving antifungal efficacy and minimizing the incidence of drug resistance. Komdima el al. reported that the application of natamycin in combination with ketoconazole is efficacious for *Aspergillus* keratitis in an animal experiment. It has been recently suggested that the combined use of natamycin and econazole eye drops yields neither additive nor toxic effects[7]. The cure rate of amphotericin B combined with fluconazole is reported to range from 81.0% to 88.9%[8,9].

In the present trial, natamycin and fluconazole eyedrops were selected due to lower toxicity compared with amphotericin B. In addition, natamycin is highly sensitive towards mycelial fungi, especially *Fusarium* and *Aspergillus*[10]. Also, fluconazole is effective against a variety of Candida and infiltrates in-

to the deep corneal layer and the anterior chamber. These advantages collectively enhance the treatment efficacy of severe fungal keratitis. Clinical observations revealed that the cure rate for superficial/moderate layer keratitis was 95.8% and the overall effective rate was 100%; the overall effective rate for those with deep layer/full-thickness keratitis decreased to 56.1% and no toxic responses were noted. Yuzhao Sun et al. utilized 5% natamycin eye drops alone to treat moderate/severe fungal keratitis after debridement. The achieved cure rates were 71% and 43%[11], smaller than achieved in previous studies. The combined use of two medicines is likely to have additive effect rather than antagonistic effect. In addition, the combined therapy of natamycin and fluconazole is an efficacious treatment against *Fusarium*, *Aspergillus*, *Penicillium* and *Mucorales*-induced keratitis while it has poor efficacy in the treatment of *Helminthosporium gramineum* infection.

In China, the incidence of fungal keratitis is constantly increasing, mainly attacking peasants. Most cases are correlated with ocular trauma of various degrees. In this study, 63% of the severe fungal keratitis cases were associated with delayed treatment. The prognosis significantly differed between the non-severe and severe groups. The combined therapy of 5% natamycin and 0.2% fluconazole was significantly efficacious for the patients in the non-severe group and yielded relatively desirable efficacy for those with deep layer corneal infection in the severe group. At present, the application of direct smear and/or fungus culture is seldom adopted for the diagnosis of fungal keratitis in Chinese grade Ⅱ hospitals, especially for drug sensitivity tests. Under these circumstances, the combined use of 5% natamycin and 0.2% fluconazole is highly recommended for rapid control of corneal fungal infections (including filamentous fungi and yeast-like fungi) and increasing cure rate of fungal keratitis.

## References


1 Wang LY, Zhang YQ, Wang YQ, et al. Spectrum of mycotic keratitis in China. China Journal of Ophthalmol, 2000,36 (2):138-140.

2 Sun XG, Wang ZQ, Luo SY, et al. Etiological analysis on the ocular fungal infection. Chinese Journal of Ophthalmology,2002,38 (7): 405-407.

3 Xie L, Zhong W, Shi W, et al. Spectrum of fungal keratitis in north China. Ophthalmology,2006,113 (11): 1943-1948.

4 Song SH, Lin YS, Lu M. The study on etiology of fungal keratitis. Chinese Journal of Practical Ophthalmology,2005,23(5):506-508.

5 Kalavathy CM, Parmar MS, Kaliamurthy J, et al. Comparison of topical itraconazole 1% with topical natamycin 5% for the treatment of filamentous fungal keratitis. Cornea,2005,24(4): 449-452.

6 Wang ZX, Wang ZQ, Luo SY, et al. Etiological analysis of ocular fungal isolates and susceptibility in vitro. Chinese Ophthalmic Research,2007,25(2):145-148.

7 Prajna NV, Praveen DVB, Nirmalan K, et al. Concurrent use of 5% natamycin and 2% econazole for the management of fungal keratitis.Cornea,2004,23 (8): 793-796.

8 Yang YD, Xu S, Zhang X, et al. Observing the therapeutic effect of injecting cornea stroma with Fluconazol to treat fungal keratitis. International Journal of Ophthalmology,2009,9 (1):154-158.

9 Zhu W, Zhang JJ. Clinical analysis on the effect to fungal corneal ulcer with flucanazole and amphotericin. J Clin Ophthalmol,2008,16(3):249-250.

10 Cui LH, Li LZ. Current progression in pharmacotherapeutics of fungal keratitis. Chinese Ophthalmic Research,2010,28(2):178-181.

11 Sun YZ, Hu YD, Chen L, et al. Effect of corneal ulcer debridement combined with antimycotic drug for fungal keratitis. International Journal of Ophthalmology,2011, 11(12):2151-2153.