A retrospective study of the spectrum of fungal keratitis in southeastern China

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Background: This study investigated the epidemiological characteristics, predisposing factors, clinical features, microbiological findings, and treatment outcomes of patients with fungal keratitis in southeastern China.

Methods: A retrospective review was carried out on 718 patients diagnosed with fungal keratitis at the First Affiliated Hospital of Fujian Medical University between January 2004 and December 2017. The sociodemographic data, predisposing factors, clinical details, microbiological findings, and treatment strategies were analyzed.

Results: Fungal keratitis was diagnosed in 718 patients (442 male and 276 female; mean age, 41.4±13.1 years). Most patients came from rural areas (79.7%) and farm work was the main occupational activity (51.7%). Cases were more common during the harvest season between October and December (41.6%). Corneal trauma (73.7%), particularly injury with vegetative matter (51.5%), was the predominant predisposing factor. Corneal scrapings obtained from 621 patients were diagnosed as positive on direct microscopy using a 10% potassium hydroxide (KOH) wet mount preparation. The positive culture rate of corneal scrapings was 89.6%. Fungal isolates were Fusarium species in 444 eyes and Aspergillus species in 98 eyes. Antifungal medications were used to treat 529 patients and 189 patients received surgery.

Conclusions: Fungal keratitis is a leading cause of infective corneal ulcers in southeastern China. Corneal trauma was the major predisposing factor and direct microscopic examination was a rapid and sensitive method for diagnosis. The species Fusarium was the most common fungal isolate. Antifungal medication was an effective method for treating early and mild cases.

Keywords: Spectrum; epidemiology; fungal keratitis; southeastern China

Introduction

Fungal keratitis is a major cause of blindness in developing countries such as China, India, Ghana, and Nepal (1-4). The increased prevalence of fungal keratitis over the past two decades is believed to be related to the growing number of cases of ocular trauma, the widespread abuse of broad-spectrum antibiotics, corticosteroids, and immunosuppressants, and the increasing use of corneal contact lenses. Long-term use of immunosuppressive agents can cause low resistance and cause fungal keratitis infection. Heightened awareness of the problem among ophthalmologists and growing familiarity with diagnostic methods have also contributed to the increased recognition of this disease.
The epidemiological features of fungal keratitis vary with respect to geographic locale and climate conditions (3). In recent years, with the continuous change of regional environment and climate, it should be a matter of concern that whether the trend of fungal keratitis and the distribution of pathogenic fungi have changed. China is a vast country, covering from tropical to sub-Arctic, this article wants to explore the fungal keratitis causes in different climate, although there are many retrospective study of fungal keratitis in China, but epidemiological data on keratitis in southeastern is sparse.

In studies undertaken worldwide, species of *Aspergillus* and *Fusarium* were found to be the predominant fungi, vegetal trauma was the main risk factor, and fungal culture of the specimen was necessary for effective therapy. A recent study in northern China by Xie et al. analyzed the epidemiological features and laboratory findings in patients with fungal keratitis (1). However, comparable data for southeastern China is poor. Southeastern China is a typical subtropical area with hilly terrain and a primarily agrarian population, which are additional predisposing factors for fungal keratitis. This study aimed to summarize the epidemiological features, laboratory findings, and treatment outcomes of 718 cases of fungal keratitis (718 eyes) in southeastern China and provide a useful guide for locally practicing ophthalmologists. We present the following article in accordance with the STROBE reporting checklist (available at https://dx.doi.org/10.21037/apm-21-1949).

**Methods**

**Patients**

This hospital-based retrospective study involved 1,463 consecutive patients (1,463 eyes) who presented with infective corneal ulcers to the inpatient department of the Eye Center at the First Affiliated Hospital of Fujian Medical University between January 2004 and December 2017. As the principal referral eye center in southeastern China, this institution serves a large proportion of patients with eye diseases in Fujian Province and neighboring provinces. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Ethics Committee of the First Affiliated Hospital of Fujian Medical University (No.: ICE-FOM-013-1.0). Individual consent for this retrospective analysis was waived. Institutional review board approval was obtained.

A detailed history from each patient with a clinical diagnosis of corneal ulcer was recorded, including the age, sex, occupation, onset of symptoms, predisposing risk factors, clinical details, prior treatment modalities, and visual outcomes. An ocular examination of both eyes was performed using slit-lamp biomicroscopy. The visual acuity at presentation, symptoms, and the size and depth of the stromal infiltrate were documented. In addition, the depth of the lesion, the presence or absence of hypopyon, and the status of the anterior chamber were evaluated.

**Diagnostic criteria**

A definitive diagnosis of fungal keratitis was made if: (I) corneal scrapings incubated with potassium hydroxide (KOH) and examined on wet mounts revealed fungal elements in smears; (II) fungus grew in more than one medium when corneal scrapings were subjected to fungal culture and strain identification; (III) histopathologic examination revealed fungal presence (1,5).

**Collection of specimens and laboratory procedures**

Corneal scrapings were aseptically collected from the leading edge or base of the ulcer using a disposable microblade, except in cases of corneal perforation. A portion of each scraping was examined microscopically for the presence of fungi, bacteria, or *Acanthamoeba* by staining with 10% KOH or Gram stain. Another portion was subjected to fungi, bacteria, or *Acanthamoeba* culture. A fungal culture was considered positive using direct microscopy, and isolates were identified based on their macroscopic and microscopic colonial morphology.

**Treatment protocol**

Antifungal medications comprising 0.5% fluconazole combined with 0.25% amphotericin B or 0.5% fluconazole combined with 5% natamycin were administered. One drop was administered every 30 minutes while the patients were awake. Patients were also treated with 200 mg of oral itraconazole daily for 21 days. If the corneal infection was not controlled or it continued to deteriorate after more than 7 days of intensive antifungal therapy, a surgical intervention was performed. Surgical interventions included therapeutic penetrating keratoplasty (PK), therapeutic lamellar keratoplasty (LK), and keratectomy in combination with a conjunctival flap.
After surgery, antifungal chemotherapeutic treatment was continued for 2 weeks, tapering thereafter. All patients were evaluated daily in the first postoperative week, once every other day in the second week, and weekly thereafter for at least 8 months. The follow-up ranged from 8 to 48 months. Each evaluation included graft clarity, late fungal recurrence, visual acuity, and intraocular pressure assessment.

**Statistical analysis**

Statistical analysis was carried out using the paired \( \chi^2 \) test and the chi square test with the software package Graphpad Prism 6.0 (Graphpad Software Inc., San Diego, CA, USA).

**Results**

**Epidemiological characteristics**

Overall, 1,463 patients with a clinical diagnosis of corneal ulcer were enrolled in this study. Of these patients, 718 (49.1%) were diagnosed with fungal keratitis and 379 (25.9%) patients had bacterial keratitis. No pathogens could be isolated in the other 279 patients (19.1%). Of the 718 patients with fungal keratitis, 442 (61.6%) patients were male and 276 (38.4%) were female. With a male-to-female ratio of 1.6:1, males were significantly more affected than females (\( P<0.01 \)). Patient age ranged from 9 to 80 years. The majority of patients were middle-aged, with the average age at presentation 41.4±13.1 years. Most patients (572; 79.7%) were from rural areas (\( P<0.0001 \)). A majority of the patients (371; 51.7%) were farm workers (\( P<0.0001 \)), and 117 (16.3%) patients were physical laborers. Fungal keratitis cases were more common from October to December (299; 41.6%), followed by July to September (182; 25.3%). The duration from the onset of symptoms to presentation to our institution ranged from 4 to 130 days (mean, 25.6±9.7 days), with most patients (529, 73.7%) presenting within 2 weeks. Before the initial visit to our institution, primary care was sought by 637 (88.7%) patients.

**Predisposing factors**

Corneal trauma (456; 73.7%) was identified as the predominant predisposing factor. Contact lens use was a risk factor in 13 (2.1%) cases. Ocular problems that predisposed patients to a corneal ulcer were present in 59 (9.5%) patients. Systemic conditions with possible immunosuppressive consequences included diabetes mellitus, pneumonia, and rheumatoid arthritis. Topical corticosteroids were prescribed to 2.6% of patients (16/619) before keratitis onset (Table 1).

**Clinical features**

The typical clinical features of fungal keratitis include a thick, dry, raised surface and stromal infiltrates with feathery margins. Satellite lesions, immune rings, hypopyon, and endothelial plaques were observed in 16%, 11%, 37%, and 9% of patients, respectively. The diameter of corneal ulcers in our cases ranged from 2 to 11 mm (mean, 5.2±1.9 mm). Corneal perforation was present in 79 (11%) patients, 198 (27.6%) patients had elevated intraocular pressure, and 47 (6.5%) patients had endophthalmitis.

**Microbiological findings**

Corneal scrapings obtained from 652 (90.8%) of the 718 eyes with fungal keratitis were positive for fungal elements on direct microscopy with 10% KOH wet mount or Gram-stained smear (Table 2).
Cultures were positive in 827 corneal ulcers. Of the 643 fungal culture-positive cases, species of *Fusarium* was the most frequent isolate [444 (69.1%)], followed by *Aspergillus* [98 (15.2%)], *Alternaria*, *Penicillium*, *Candida albicans*, *Microsporum*, and *Sporothrix schenckii*. There were also 22 unidentified cultured specimens (Table 3).

### Treatment outcome

Antifungal medications were used to treat 529 patients while surgery was used in 189 patients, including therapeutic PK in 61 (32.2%) patients, therapeutic LK in 35 (18.5%) patients, keratectomy in combination with a conjunctival flap in 67 (35.4%) patients, and evisceration in 26 (13.8%) patients. Follow-up ranged from 8 to 48 months. Halfway through treatment, 21 patients were lost to follow-up.

Combined antifungal medications were effective for early and mild cases of fungal keratitis. Of the cases cured with medication, the course duration ranged from 19 to 96 days (mean, 27±8.9 days), showing therapeutic effect within the first 5 to 14 days. Secondary glaucoma occurred in 38 cases, requiring trabeculectomy. A corneal leukoma formed in 216 cases (40.8%), a corneal macula formed in 192 cases (36.3%), and an adherent leukoma formed in 43 cases (8.1%).

Fungal infection recurred in 4 (11.4%) of the patients who received LK. The recurrent keratitis was controlled with PK, and the grafts remained clear at follow-up. Of the cases receiving PK, keratitis recurred in 9 (14.8%)
patients. Repeat grafting was performed for 2 of these cases, 3 required conjunctival flaps, 3 cases eventually required evisceration for control of the disease, and one was lost to follow-up.

Discussion

The incidence of fungal keratitis in our study was similar to that reported in northern China (1). Research results from different parts of the world indicate that the incidence of fungal keratitis ranges from 6% to 56% (6,7). A Taiwanese study found that fungal pathogens were isolated from only 13.5% of 476 eyes with microbial keratitis (8). This disparity could be due to the use of more mechanized production modes in Taiwan compared to those used in southeastern China.

Farmers were the most common patients with fungal keratitis (1-3). In our study, 51.7% of patients were farm workers and most patients were from rural areas. This corresponds to the sex and age of patients in this study. In southeastern China, particularly in Fujian province, agriculture is the primary industry and the labor force mainly comprises young to middle-aged men. Correspondingly, fungal keratitis cases were more common between October and December due to the high humidity and temperature difference between day and night during these months. This type of environment results in a propensity for fungal growth. Farm workers are often very busy during the harvest season and are prone to vegetative injuries.

Early diagnosis and early treatment are critical for the treatment of fungal keratitis (9). In our study, most patients visited the hospital within 15 days after the onset of symptoms, indicating that in southeastern China, patient health awareness had significantly improved with rapid economic development. We found that the longer the duration of symptoms and the longer the delay to obtaining medical care, the worse the response to antifungal agents, the more likely it was that patients underwent corneal surgery, and the greater likelihood of sequelae. The differential diagnoses include bacterial keratitis. Bacterial keratitis is usually caused by bacterial infection after trauma. There are many kinds of Species of pathogenic fungi in fungal keratitis, among which fusarium and aspergillus are the most common. Bacterial keratitis is a urgent disease, which occurs usually between 24 and 48 hours after the occurrence of trauma, the common symptoms are redness, photophobia, tears, decreased vision, increased secretion, etc. Fungal keratitis is slower, but with higher blinding rate. The patient’s corneal infiltration can easily cause endophthalmitis.

Corneal trauma was clearly identified as the predominant predisposing factor in our study. This is similar to the findings from northern China and from many agricultural producing countries, such as India and Nepal (1,2,4). In previous studies, the percentage of corneal trauma has been reported to range from 8% to 66% (6,10), with agricultural vegetation identified as the most frequent cause of injury (44% to 69% of cases) (6,9,11). Contact lens wear was reported to be a major risk factor in many studies (2,12) but was much lower in our study as farmers in China seldom wear contact lenses. Topical corticosteroid use was also reported to be correlated with the incidence and course of fungal keratitis (2,13,14). In our study, those patients who used topical steroids had more extensive infiltrates and the response to antifungal therapy was slower. Therefore, we agreed that corticosteroids should be contraindicated in patients with fungal keratitis (5,13,15).

In the present study, the positive rate of fungal filaments on direct microscopic examination was 90.8%, which is consistent with previous studies from northern China (88.7%) (1) and Northwest India (90%) (11). The sensitivity of 10% KOH wet mount preparation was higher than the sensitivity of a Gram-stained smear in this study, which is similar to previous reports (11,16). We concluded that direct microscopic examination with a 10% KOH wet mount should be one of the main methods for early diagnosis of fungal keratitis; it is convenient, rapid, inexpensive, highly sensitive, and highly specific. We also believe it is important for doctors to master specific skills for corneal scraping, which include removing the surface secretions and necrotic tissue first, then repeatedly scraping the corneal ulcers at the bottom and edge, and finally smearing a thin layer on a wet mount. This helps to show the fungal hyphae, spores, and Acanthamoeba more clearly.

The positive culture rate in corneal scrapings was 89.6% in this study, which is similar to previous studies (1,3). A fungal culture is considered the ‘gold standard’ for the diagnosis of fungal infections. However, this process often requires more than 5 days and thus is not useful for early diagnosis. Patients with a suspected case of fungal keratitis should simultaneously have a direct microscopic examination and a fungal culture to avoid a delayed diagnosis.

The most common fungus reported in China is Fusarium, which accounts for 58.7% of cases, similar to the
findings of our study (1,2). The climate of Southeastern China is different from that of northern China, and there is no obvious difference between the seasons. The temperature and humidity are relatively higher, which can contribute to the growth of fungi. We want to explore the causes of fungal keratitis in different climates, but the data show that the main pathogenic bacteria is Fusarium. In this study, Fusarium was the most frequent isolate, followed by Aspergillus. Fusarium has also been found to be the predominant species in Southern India, Florida, Paraguay, Nigeria, Tanzania, Hong Kong, and Singapore (6,7,11,13,17-22). However, in Northern India, Nepal, and Bangladesh, Aspergillus is the most frequent cause of fungal keratitis (2,4,23). This phenomenon may be explained by the differences in demographic characteristics, climate, and the natural environment. Filamentous fungi are more frequently encountered than yeasts in tropical and subtropical regions. Fusarium grows in cornea horizontally, diagonally and vertically, and the hyphae are easy to spread and multiply around the lesion while Aspergillus grow in cornea diagonally and vertically. Filamentous fungi can penetrate corneal stroma and into anterior room, invade iris and the tissue inside the eye, cause fungal iridocyclitis, intraocular inflammation, and complicated cataract. Fusaria are the most common pathogenic bacteria, and the peak onset periods were during autumn and summer harvests. Fully understand the epidemiological characteristics of fungal keratitis, early diagnosis and early treatment are of great significance for prognosis.

Conclusions

In summary, this study focused on the epidemiological and etiological data obtained from a large cohort of patients with fungal keratitis in southeastern China and provides significant insights into the understanding of this potentially devastating corneal disease. In southeastern China, the Fusarium species was the most commonly isolated pathogen, agricultural activity was the principal causative factor, and a large proportion of patients received early treatment and were cured by antifungal medications. We hope that our study may help our colleagues diagnose and properly treat this disease and achieve a better outcome for patients.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://dx.doi.org/10.21037/apm-21-1949

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**Ethical Statement:** The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Ethics Committee of the First Affiliated Hospital of Fujian Medical University (No.: ICE-FOM-013-1.0). Individual consent for this retrospective analysis was waived.

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